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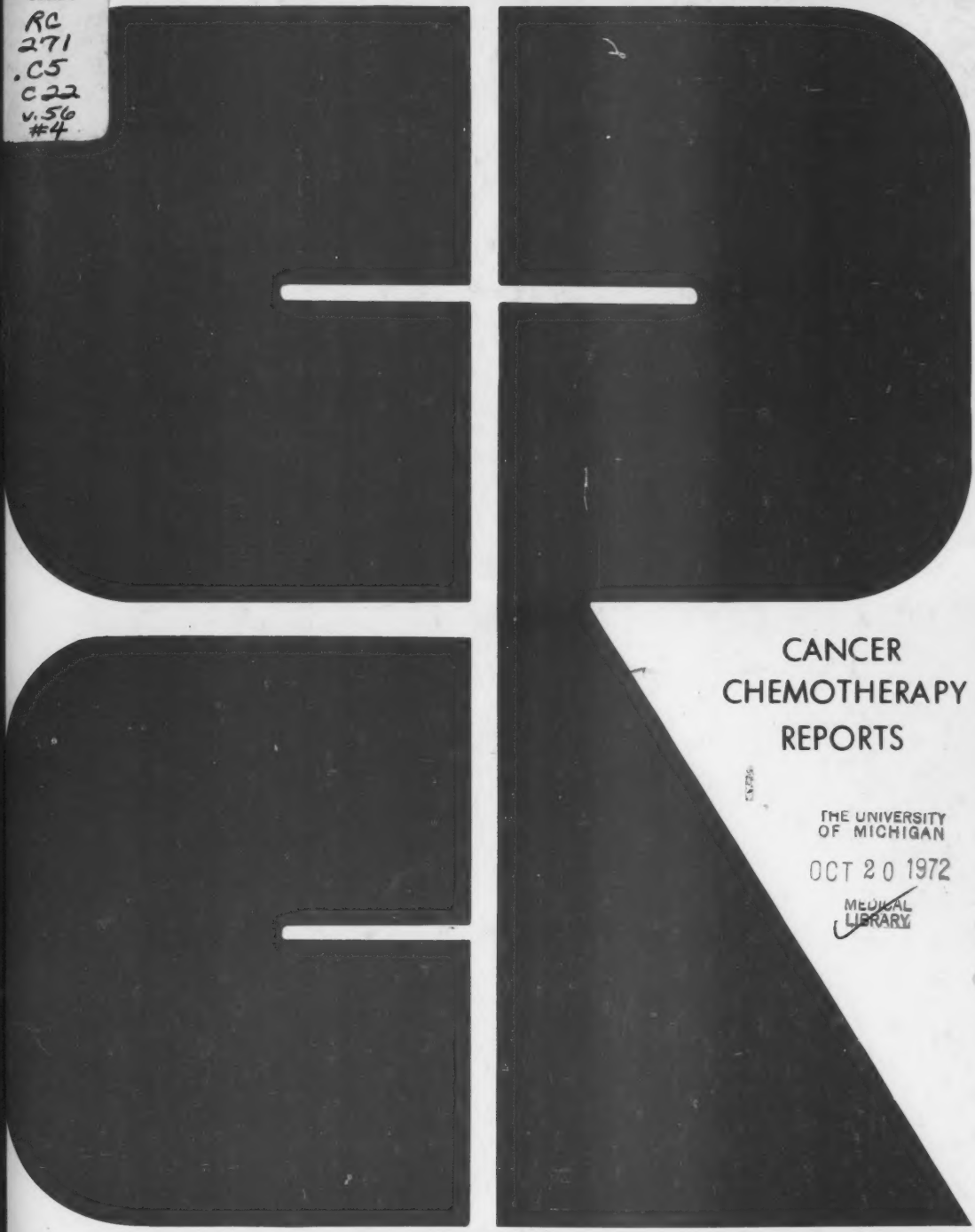

CANCER CHEMOTHERAPY REPORTS

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August 1972
Vol. 56, No. 4

Cancer Chemotherapy Reports

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LETTER

On the Reported Lack of Effectiveness of Daunomycin (NSC-82151) and Adriamycin (NSC-123127) on Solid Tumors

After the initial reports by Di Marco and coworkers on the activity of daunomycin and adriamycin (1,2) in a spectrum of solid and ascitic tumors, a number of papers were published on the antitumor activity of these antibiotics in several tumor systems. From the data available in the literature it appears that significant activity has been described for both drugs used in the intraperitoneal (ip) treatment of ascitic tumors and leukemias only. Several papers reported that treatment with daunomycin and adriamycin does not induce significant impairment of tumor growth or increase in survival time of solid tumor-bearing animals in systems such as solid Sarcoma 180 (3) and spontaneous [1] or transplanted (4) mammary carcinoma. Since these results contrast with observations in this laboratory, ie, marked activity of daunomycin and particularly adriamycin in such systems (1,5), it seems appropriate to mention a few points which may account for the observed differences in activity.

In our laboratory, treatments have been performed whenever possible by the intravenous (iv) route, and when this is not possible we have used the subcutaneous route. In the other laboratories, the ip route has been utilized.

Both daunomycin and adriamycin induce marked local toxic effects: when injected ip, they induce peritonitis and peritoneal adhesions resulting in increased toxicity compared to the iv route. For instance, the acute LD50 for daunomycin is 26 mg/kg when given iv and about 10 mg/kg when given ip. When given for 8 consecutive days, the maximal tolerated dose of daunomycin drops from 3.0–3.5 mg/kg/day for the iv route to 1.00–1.25 mg/kg/day for the ip route. Similar results can be obtained for adriamycin. Moreover, peritoneal irritation and adhesions may account for late toxic deaths induced by ip treatment with these antibiotics. The total dose which can be given is therefore much smaller when these drugs are given by the ip route. These smaller doses result in lower drug levels in the tumor tissue (6)[1] which may account for the reported lack of activity. Moreover, local peritoneal irritation may result in poor drug absorption after repeated ip treatments.

From these considerations it would appear that the iv route of administration should be recommended, especially in the case of slow-growing tumors such as mouse mammary carcinoma, which must be treated over a long period of time for optimal results (5). In our experience (L. Lenaz and A. Di Marco, unpublished results) daunomycin and adriamycin given iv according to appropriate schedules may result in a marked impairment of tumor growth and an increase in average survival time in spontaneous or advanced transplanted mammary carcinoma. A small number of "cures" (10%–20%) has also been observed; such a result has never been observed by others (4) with a variety of antitumor agents. On the other hand, ip treatment of ascitic tumors may give better results than iv treatment, since initial high drug concentrations may be obtained in the tumor cells.

[1] Balconi, G., Bossi, A., Donelli, S., et al. Chemotherapy of a spontaneous mammary carcinoma in mice. Relation between in vitro-in vivo activity and blood and tumor concentrations of several antitumoral drugs. To be published.

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May 24, 1972

COMMENTARY: Strategy in Drug Design [1,2]

Corwin Hansch [3]

SUMMARY

The game of drug modification continues to become increasingly expensive to play. The synthesis and testing of derivatives of compounds active against cancer is increasing as more and more new leads are found. For each new lead compound, possibly millions of derivatives can be synthesized. How does one select a well-balanced set of derivatives for synthesis and testing? The use of substituent constants and regression analysis helps to avoid redundancy in the choice of derivatives. It also aids in planning research so that the proper derivatives are selected for separation of electronic and hydrophobic effects of substituents.

[Cancer Chemother Rep 56:433–441, 1972]

The major problem in medicinal chemistry, once a new molecule with some desirable pharmacologic activity is found, is to systematically modify the molecule so that by making the fewest possible number of derivatives one can obtain the largest number of new compounds with maximum potency and minimum toxic side effects. In carrying out such an operation, one hopes to achieve some understanding of the relationship between chemical structure and pharmacologic activity. This latter goal is not always achieved. The reasons for failure in this area are more easy to understand than the reasons for success. In modifying a parent molecule, one is simultaneously making a variety of changes which may or may not be varying in a linear mode. The most important of these are hydrophobic, electronic, and steric changes (1,2). Changes in these various properties not only affect the primary potency of the members of a set of congeners but, in addition, may have pronounced effects on the way the congeners are metabolized. And, of course, in a system as complex as a mouse there are a variety of ways in which metabolism may occur. While metabolism usually results in lower or complete loss of activity in the metabolite, there are many well-documented cases where a molecule of much greater activity has resulted from "metabolic activation" of a parent compound.

In addition to the above formidable problems, the medicinal chemist must try to decipher how modification of a parent molecule may affect the adsorption, distribution, and elimination of the various derivatives. What guidance have the years of study of structure-activity relationships placed at our disposal to help in the decision-making process of selecting the most likely derivatives for synthesis and pharmacologic testing?

Ellipticine [4] (fig 1), which has some activity as an antineoplastic agent, is a good model for discussing strategy in drug design. How should one go about modifying this compound to increase its value as an anticancer drug? Omitting the two nitrogen positions, there are nine ring positions at which we might make substitutions. One can easily think of 100 functions to test in these positions. Considering all possible combi-

[1] Received Apr 10, 1972; accepted Apr 13, 1972.

[2] Supported by grant CA-11110 from the National Cancer Institute, National Institutes of Health.

[3] Department of Chemistry, Pomona College, Claremont, Calif.

[4] Ellipticine: NSC-71795; 6*H*-pyrido[4,3-*b*]carbazole, 5,11-dimethyl-.

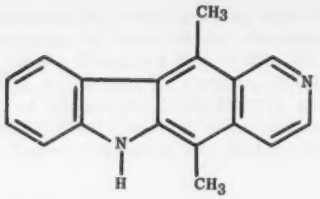

FIGURE 1.—Chemical structure of ellipticine.

nations, this would mean 100^9 possible derivatives. Considering only ten functions, we have the formidable figure of 10^9 possible derivatives. Of course we could make substitutions on either or both of the nitrogen atoms which would greatly increase the possibilities, or, to make matters still more complicated, we might consider more hetero atoms in the rings (oxygen, sulfur, or more nitrogen atoms would be interesting). Then, of course, the hetero atoms could be moved around the ring to yield a bewildering variety of heterocycles which could be substituted in many ways with many substituent[s]. The possibilities that modern synthetic techniques have opened up for us completely boggle the mind.

John Topliss of the Schering Corporation has recently pointed out [5] that there are two limiting types of situations the drug designer faces. In the type I situation, the chemist can prepare derivatives relatively fast compared to the pharmacologist's testing procedure, which might require 6 months to a year or more before the activity of a given derivative can be specified. Under these circumstances the medicinal chemist must design and make a set of congeners, submit them for testing, and await the results. In the type II situation, pharmacologic testing is fast compared to synthesis time. In these circumstances, feedback from the pharmacologist is available for each new synthesis. These two extremes call for different kinds of strategy in drug modification.

TYPE I SITUATION

Considering first the type I situation, how should one prepare a *proper* sample of the billions of possible derivatives of ellipticine to test? This is an extremely difficult problem in decision making, partly because there are so many different positions to consider for substitution, and partly because the synthesis of many of the derivatives would be difficult and expensive. The first decision is, how many derivatives can one afford to make in the first set to be submitted for testing? Let us assume that for the ellipticine ring cost of synthesis is no problem and that derivatives can be made relatively rapidly. Let us also assume that enough manpower is available so that in a few weeks 16 molecules can be made and sent in for testing which will require 6 months for results. What variations in the molecule should be made to obtain maximum information which can be used to design still better derivatives?

Substituent effects will be factored into three main categories (1,2) which will be considered separately: hydrophobic, electronic, and steric.

In studying ellipticine, of course, one should confirm whether either or both of the methyl groups are essential for activity. While it is highly unlikely that the methyl group per se would be a potent pharmacophoric function, aromatic and allylic methyl groups are easily oxidized in animals to the CH_2OH function. This function appears in many drugs (3). For example, such oxidation may produce the active form of tetrahydrocannabinol. For our present discussion, the methyl group will be assumed to be unessential. If a methyl group is essential, the following discussion would simply be modified by including a methyl function in the parent structure.

Hydrophobic Effects

Two types of hydrophobic effects are involved: specific and nonspecific. Nonspecific hydrophobic effects mainly determine the distribution of the drug via a random walk process in the test animal. The log P value of the drug is a good relative measure of the nonspecific hydrophobic character. The parameter log P_o is used to designate the ideal lipophilic character for a set of congeners (4). Since log P_o is usually found in the range of 0–6, the set of substituents used to modify ellipticine should more or less encompass this range. The log P for the

[5] Joint meeting of the American Society of Pharmacology and Experimental Therapeutics and the Medicinal Chemistry Division of the American Chemical Society. Burlington, Vermont, Aug 1971.

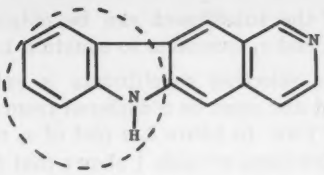

FIGURE 2.—Chemical structure of the parent molecule of ellipticine.

parent molecule must be measured or calculated in order to know which substituents to choose. Log P for the parent compound (fig 2) may be estimated as follows:

$$\pi_{C_6H_5NH-} + \log P_{\text{isoquinoline}} = 1.37 + 2.08 = 3.45.$$

$\pi_{C_6H_5NH-}$ is calculated as follows: log $P_{C_6H_5NHC_6H_5}$ − log $P_{C_6H_6}$ = 3.50 − 2.13 = 1.37. Log P for desmethylellipticine has been measured and its value is 4.06. Subtracting 0.50 (π_{CH_3}) from this, we obtain a calculated value for the parent compound of 3.56 which agrees with the above calculated value. Assuming a log P value of 3.5 for the parent molecule, we should consider substituents with π values ranging from about −3.0 to +1.5. This additive constitutive character (5) of log P enables one to plan carefully before starting any synthesis.

There are three quite different types of positions on the parent molecule (fig 2): the indole nitrogen, the pyrido nitrogen, and the carbon positions. If we elect to substitute carbon positions, we might consider the substituents of table 1. The parent molecule is so large that it would be best to study it in sections; that is, steric, electronic, and even hydrophobic effects of substituents on the indole ring might well be much different from those on the pyridine ring. If the indole ring is selected for study, it would be best (assuming nothing is known about substituent effects) to choose the 5- and 6-positions for the first set of drugs. In this way we could avoid the problem of proximity effects from the 7-position on the indole nitrogen atom. If eight substituents from table 1 were placed in the 5-position and another eight in the 6-position, we should be able to sketch out equation 1 (ie, obtain first approximations for the parameters $[k_1 - k_4]$):

$$\log 1/C = -k_1\pi^2 + k_2\pi + k_3\sigma + k_4. \qquad (1)$$

From equation 1, π_0 could then be calculated from which we could estimate log P_0 as follows: π_0 + 3.50 = log P_0. In order to check whether

TABLE 1.—Hydrophobic and electronic substituent constants

Constant	Function	π	σ_p	σ_m
1.	$-CO_2^-$	−4.36	0.00	−0.10
2.	$-SO_2NH_2$	−1.82	0.57	0.46
3.	$-SO_2CH_3$	−1.63	0.72	0.60
4.	$-CONH_2$	−1.49	0.36	0.28
5.	$-NHCONH_2$	−1.30	−0.24	−0.03
6.	$-NHCOCH_3$	−0.97	0.00	0.21
7.	−OH	−0.67	−0.37	0.12
8.	−CN	−0.57	0.66	0.56
9.	$-OCH_3$	−0.02	−0.27	0.12
10.	−H	0.00	0.00	0.00
11.	−F	0.14	0.06	0.34
12.	$-CH_3$	0.50	−0.17	−0.07
13.	−Br	0.86	0.23	0.39
14.	$-C_2H_5$	1.00	−0.15	−0.07
15.	$-SCF_3$	1.44	0.50	0.40
16.	$-C_6H_5$	1.96	−0.01	0.06
17.	$-OC_6H_5$	2.08	−0.32	0.25

there are any specific hydrophobic or possibly steric effects, one should fit the data for the 5- and 6-substituted derivatives separately to equation 1. If the constants k_1–k_4 are approximately the same for each of these fits, one can assume the absence of specific hydrophobic effects for these two positions (6).

If we are lucky and steric effects of substituents do not intervene and if metabolism of the various derivatives varies linearly with π or is constant, then we should be able to evaluate possibly the most powerful parameter (log P_0) for decision making regarding positions on the other rings of the parent molecule. If reasonable confidence limits can be placed on log P_0, then enormous numbers of derivatives with highly unfavorable log P values can be eliminated from consideration with some rational justification.

Electronic Effects

With the proper choice of substituents, information about the sign as well as the magnitude of k_3 in equation 1 should also be obtained. In addition to ascertaining whether electron-releasing or electron-attracting substituents promote activity, information on the positional effect of the substituent can be obtained by fitting σ_m and σ_p constants to equation 1.

Care in selecting substituents is extremely important and must be considered from several points of view. In figure 3, a plot of σ_p versus π for the functions of table 1 shows that for this set, σ_p and π are independent variables and it is possible to assign separate roles (electronic and hydrophobic) to this set of functional groups. Unless attention is given to this problem, one can easily select a set of substituents for σ and π which are linearly related so that it is almost impossible to discern whether the potency is a function of the electronic or hydrophobic effect of the substituents. This is illustrated in figure 4, which is based on the data of table 2. At first glance, the set of functions in table 2 seems quite diverse in nature, and this is true as far as their σ or π values alone are concerned. If we were only interested in one of these functions, the set would be quite satisfactory. It is only when we wish to separate electronic and hydrophobic effects that we run into difficulties. A more detailed analysis of this problem of cocorrelation by various physical parameters has been made by Paul Craig (7).

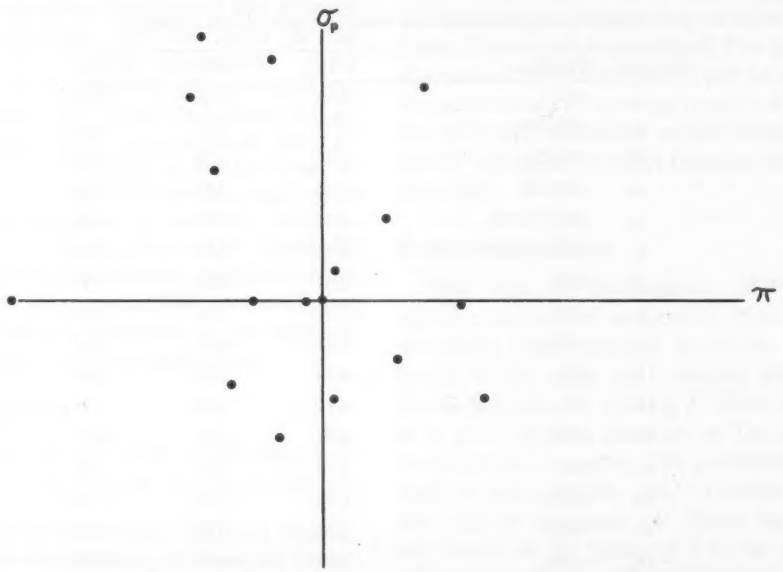

FIGURE 3.—Plot of σ_p vs π values for the functions shown in table 1.

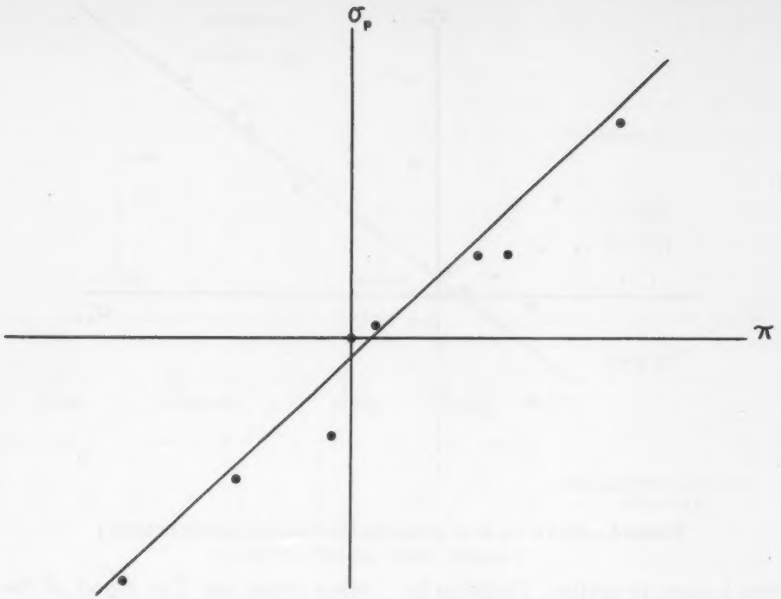

FIGURE 4.—Plot of σ_p vs π values for the functions shown in table 2.

The same kind of problem must be faced in attempting to separate the positional nature of the electronic effect. In figure 5, a plot of σ_m versus σ_p values for the functions of table 1 is given. The scatter here is not as broad as in figure 3, indicating the greater difficulty in separating the electronic positional role of substituents. The line in figure 5 indicates the high correlation between σ_m and σ_p values for a subset of nine of the 16 functions. It is apparent that greater care must be given to planning which derivatives to make if one wishes to obtain the maximum amount of insight possible in structure-activity relationships.

TABLE 2.—Hydrophobic and electronic substituent constants

Constant	Function	π	σ_p
1.	$-NH_2$	−1.23	−0.66
2.	−OH	−0.67	−0.37
3.	$-OCH_3$	−0.02	−0.27
4.	−H	0.00	0.00
5.	F	0.14	0.06
6.	Cl	0.70	0.23
7.	Br	0.86	0.23
8.	SCF_3	1.44	0.50

In none of the above plots has the $-COO^-$ function been included. This is not a well-behaved function because of its partially ionized character under physiologic conditions. Although it is useful in a qualitative sense to feel out structure-activity relationships, the very large negative π value for the $-COO^-$ function often makes molecules too hydrophilic. The most important functions of table 1 for exploring the positional nature of the electronic effect are 5, 7, 9, 11, and 17.

Since the parent molecule of ellipticine is quite lipophilic, the substituents of table 1 have been chosen with a bias for negative π values. If the parent molecule under study was much more hydrophilic, then one would want to choose more substituents with higher π values.

The π values of table 1 are from the benzene system and were obtained as follows:

$$\log P_{C_6H_5X} - \log P_{C_6H_6} = \pi_X.$$

While π is more or less constant, it does vary

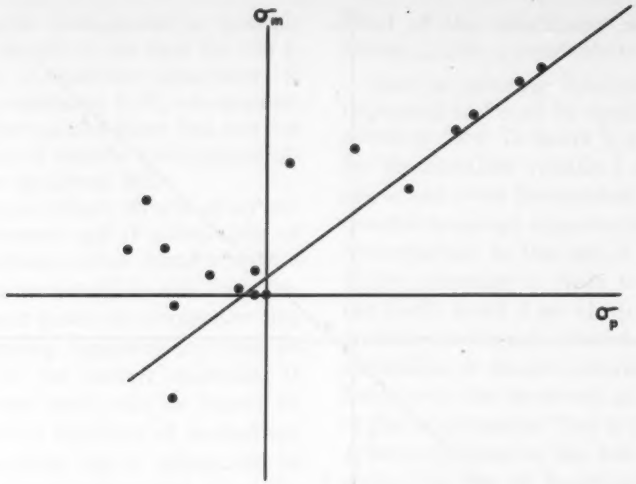

FIGURE 5.—Plot of σ_m vs σ_p values for the functions shown in table 1.

somewhat from system to system. Variation is largely dependent on the electronic character of the region in which the substituent is placed (5). For example, π_{NO_2} from the benzene system is –0.28, while π_{NO_2} from the aniline system is 0.49. It has been shown that the difference in π values obtained from a number of systems compared to the benzene system (π aniline system $-$ π benzene system $= \Delta\pi$) is related as follows:

	n	r	s	
$\Delta\pi_X = 0.90\sigma_X + 0.02$	11	0.974	0.079.	(2)

The above discussion has been limited to the use of σ for modeling electronic effects. In a more thorough study of electronic effects one would also want to consider other electronic parameters (2) such as σ^+, σ^-, σ_I, σ_R, and $\sigma^\cdot$.

TYPE II SITUATION

The type II situation, where synthesis of derivatives is slow compared to pharmacologic testing, allows the drug designer to use feedback from the test on each derivative in the design of the next. Drug modification becomes much like a game of chess in which one makes a move, obtains a response, and then makes another move, etc. The object of the game is to find the most potent members of the family with the fewest possible moves.

John Topliss [6] has shown that the type II case leads to the branched network of scheme I (fig 6). The use of substituent constants in such a "Topliss Tree" can be of great help in the decision-making process of drug design. In scheme I, the symbols –, 0, and + under each derivative indicate that the derivative may be less active, equally active, or more active than the parent molecule. The case where the change in relative biologic response is zero (ΔRBR = 0) is only important in those few instances where substituents have little effect on the activity of the parent function. These are special cases. To simplify the present discussion, the case where ΔRBR is 0 will not be considered.

The 5-bromo derivative is suggested in scheme I as a good opening gambit. A long tradition of success in medicinal chemistry using halogens prompts this move. An underlying reason for this is the stability of these functions to metabolism. The halogens are also well-behaved functions as far as the substituent constants σ, π, and E_s are concerned. Bromine is suggested rather than chlorine because it is a bit more lipophilic and because bromine derivatives are, in general, a little easier to make.

[6] *See* footnote 5.

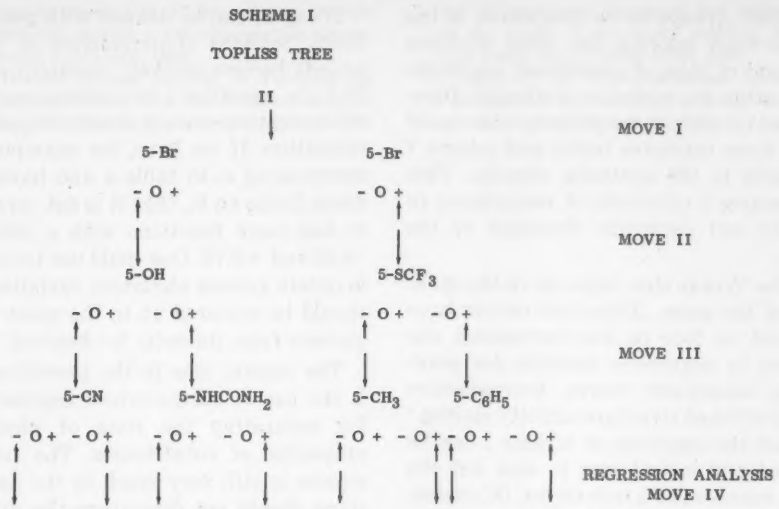

FIGURE 6.—Topliss Tree: scheme I.

The introduction of bromine in Move I yields a derivative which is either more or less active. Consider first the case on the right (fig 6) where the small arrow on the + indicates an increase in RBR. What should the next move be? An increase in activity may be due to the $+\sigma$ or the $+\pi$ effect of bromine; or, it may be that both the $+\sigma$ and $+\pi$ effects (electron withdrawal and increased lipophilic character) combine to produce greater activity. It is rare that an increase could be due to a positive steric effect of the large bromine although this possibility cannot be ruled out completely. To test these possibilities we can introduce a function with more of the same SCF_3. Both π and σ are larger for this function than for bromine, and, of course, it is a more bulky group (greater steric effect). If the 5–SCF_3 derivative is more active than the 5-bromo derivative, we can introduce a still more lipophilic group, the phenyl function. This function does not have a high σ value so that if ΔRBR is again +, it must be because of the importance of the lipophilic character of the function. It would be surprising if a function more lipophilic than the phenyl function would produce an increase in ΔRBR since the 5-phenyl derivative would have a log P value of 5.5; neutral drugs with log P values greater than 5 are extremely rare. To check this possibility one could add to the phenyl ring (eg, 5-[4-Br-C_6H_4]), or long aliphatic chains (amyl or hexyl) could be used.

If the 5-SCF_3 derivative has a $-\Delta$RBR, this could be because its log P is greater than log P_o or because its rather high σ value outweighs a beneficial π value. Making the 5-CH_3 derivative would yield a derivative having a $-\sigma$ value and lower lipophilic character.

Consider now the left branch of scheme I (fig 6) where ΔRBR for the 5-bromo derivative is –. This might be due to the fact that log P for this derivative exceeds log P_o or that an electron-releasing function is needed instead of the electron-attracting bromine. The 5-OH function would be useful in checking these possibilities. If ΔRBR is + for 5-OH, we could check a still more water-soluble electron-releasing function, 5-$NHCONH_2$. If ΔRBR for 5-OH is –, it could be that electron release is bad but that we do need a function with a $-\pi$ value. The 5-CN would be useful in testing this possibility.

This Topliss Tree is by no means the only opening gambit in the modification of the parent molecule; it is merely suggestive. The actual derivatives selected for preparation in each move must depend on the judgment of the syn-

thetic chemist. Always to be considered is the trade-off between making the ideal function from the point of view of substituent constants and going after an easier-to-synthesize function. The use of table 1 (in practice one would use a much more extensive table) and scheme I can be helpful to the synthetic chemist. This approach assures a minimum of redundancy in the lipophilic and electronic character of the derivatives.

The Topliss Tree is most valuable in the opening moves of the game. After test results have been obtained on four or five derivatives one should resort to regression analysis for guidance in the subsequent moves. Retrospective analysis of published structure-activity studies [7] indicates that the approach of scheme I can be a most direct and logical way to seek out the most active members of a new series. Of course, the neat logical approach outlined above will by no means always be successful. The intervention of severe steric or metabolic factors could completely mask the interpretation of the electronic or lipophilic action of the substituents. However, even though one cannot always rationalize structure-activity relationships in terms of σ, π, and E_s, it is still advantageous to start with this assumption. If the situation does lend itself to such treatment, drug design is enormously aided; if not, *nothing is lost.*

The general approach outlined above might be termed conservative; that is, the functions for which π and σ constants are available are limited and these are the more simple functions. The argument for studying these first is that if log P_0 and k_3 of equation 1 can be even roughly evaluated, these constants can serve as very important guidelines in making less conservative changes in the parent molecule. We have by no means approached the state where everything can be calculated by computers. The drug designer will want to try a variety of hunches in modifying the parent molecule based on intuition gained from past experience. The approach outlined here is certainly not meant to discourage this traditional method which has produced so many excellent drugs; rather, it is meant to supplement intuition.

[7] *See* footnote 5.

If log P_0 can be defined with good confidence limits, millions of derivatives of parent compounds become unlikely possibilities for study. If k_3 in equation 1 is positive, most functions with negative σ values can be dropped from consideration. If we have, for example, tested the extremes of σ_p in table 1 and have good confidence limits on k_3, then it is not very interesting to test more functions with σ values between −0.32 and +0.72. One could use two substituents to obtain greater electronic variation. This path should be explored up to the point where a departure from linearity is observed.

The central idea in the preceding discussion is the use of computerized regression analysis for separating the roles of physicochemical properties of substituents. The fact that this science is still very much in the developmental stage should not discourage the drug designer from employing such techniques. When quantitative pharmacologic test data are available and substituents with known constants have been employed, computer analysis of the results is so easy and inexpensive that the use of this tool should not be neglected. The costs will usually be much less than the cost of synthesis of one derivative.

One hopes that in the next decade we shall be able to formulate some standard opening gambits for the rational modification of newly discovered lead compounds. It is high time we started thinking more concretely about the problem. Topliss has opened in an imaginative way what promises to be an interesting conversation.

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ET AL. Passive permeation of organic compounds through biological tissue: a non-steady-state theory. Mol Pharmacol 5:333–341, 1969.

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7. CRAIG, P. N. Interdependence between physical parameters and selection of substituent groups for correlation studies. J Med Chem 14:680–684, 1971.

Quantitative Structure-Activity Relationships of Antineoplastic Drugs: Nitrosoureas and Triazenoimidazoles [1,2]

Corwin Hansch,[3] Nelson Smith,[3] Robert Engle,[4] and Harry Wood [4]

SUMMARY

The structure-activity relationships of nitrosoureas and imidazole carboxamides in the treatment of L1210 leukemia in mice are examined. The activity of each type of drug is parabolically related to lipophilic character. Lipophilic character is operationally defined by log *P* where *P* is the octanol-water partition coefficient of the drug. Optimum lipophilic character for the nitrosoureas occurs at log *P* values near −0.6. The LD10s for the nitrosoureas are also parabolically related to log *P*, with maximum toxicity occurring at a log *P* value of 0.4. This suggests that a study of more hydrophilic nitrosoureas should uncover more potent, less toxic drugs. There is some evidence that this finding may also apply to other alkylating agents. The activity of the imidazole carboxamides shows a much different dependence on lipophilic character. The optimum log *P* value for this series is about 1.
[Cancer Chemother Rep 56:443–456, 1972]

There are two main problems concerning the search for drugs active against cancer. The first is that of uncovering new pharmacophoric functions, and the second is that of refining and tuning these basic functions to obtain the most potent drugs with the fewest possible side effects. Research strategy must be quite different for each of these problems. Since there is so little in the way of theory to guide us in the search for qualitatively new pharmacophoric functions, rapid screening of the largest possible number of *truly new* chemical types is of the utmost importance. Once an active molecule is found, the medicinal chemist and the biologist have a new problem. Carefully planned structures must be made and carefully tested. The medicinal chemist must take note of all of the significant structure-activity relationships, toxic as well as therapeutic, which is a very large order. The biologist is responsible for establishing the rate and quality of progress in refining pharmacophoric functions. The quality of his results will spell success or failure.

The purpose of this report is to consider the use of computerized regression techniques for obtaining insight into the important physicochemical parameters which determine the activity and toxicity of two types of antitumor drugs.

First let us consider the magnitude of the problem, using as a representative molecule that of the quite active nitrosoureas (fig 1). Montgomery et al (1, p 703) have pointed out the potential of these compounds, especially concerning their apparent ability to cross the so-called blood-brain barrier.

In the structure shown in figure 1 there are seven hydrogens which might be replaced by

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[2] Supported in part by contract PH43-NCI-C-2112 from Division of Cancer Treatment (DCT) (formerly Chemotherapy), National Cancer Institute (NCI), National Institutes of Health.

[3] Department of Chemistry, Pomona College, Claremont, Calif.

[4] Drug Development Branch, Drug Research and Development, DCT, NCI.

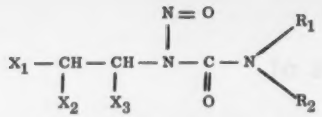
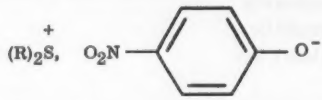


FIGURE 1.—Parent structure of the nitrosoureas.

various substituents. If we consider trying ten possible substituent changes at five of these positions, the total possible number of variations would be 10^5. Actually, the number would be three times this large if we also replace the carbonyl oxygen with sulfur and nitrogen. Of course, ten is a small number of changes to consider in some places and a rather large number in others. For example, it appears that X_1 must contain a good leaving molecule, such as fluorine, chlorine, bromine, iodine, or $ROSO_2$. This limits the possibilities for substitution somewhat, but there are many aliphatic and aromatic sulfates and sulfonates that one could try as well as more esoteric functions such as:



Also, in general, it seems that either R_1 or R_2 must be hydrogen, since two substitutions destroy all activity. Considering changes in only one of the R groups, however, it is easy to think of 100 or even 1000 variations. The point to be made is that there are literally millions of variations of the parent nitrosourea structure which can be made. Since it is not practical to make and test more than a few hundred or, at most, a few thousand variations, how does one select a reasonably representative sample? Before this problem is considered in more detail let us consider some results with selected sets of congeners.

METHODS

All of the equations derived in this report have been obtained via a nonweighted least squares fit.

Calculations of log *P* values for many of the derivatives shown in tables 1 and 2 have been based on a measured log *P* value of 2.83 for *X* = chlorine, *R* = cyclohexyl. To calculate the values for fluorine analogs we have used the difference of 0.60 between π values for chlorine and fluorine taken from the aliphatic compounds *RX* (2). These calculated values are supported by the measured values in table 1. The difference in the measured log *P* values between compounds 14 and 17 is 0.64, and the difference between the measured log *P* values for compounds 8 and 16 is 0.54. This additive-constitutive character of log *P* and π can be of great help in the design of drugs. Using the octanol-water reference scale, one can design molecules having a given degree of lipophilicity with considerable confidence (3).

To calculate log *P* for *t*-butyl and methyl derivatives we have used π_X values of 1.68 and 0.50 respectively. For 4-acetyloxycyclohexyl derivatives we have used a π value of –0.27; this value was calculated by subtracting the π value for two methyl groups (2 × 0.50) from 0.73, the log *P* for $CH_3COOCH_2CH_3$ (2). Log *P* for the adamantyl derivative was calculated as follows:

$$\text{Log } P_{\text{1-hydroxyadamantane}} - \pi_{OH} = 2.14 - (-1.16) = 3.30 = \pi_{\text{adamantyl}}$$

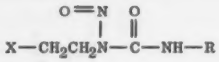
$$\pi_{\text{adamantyl}} - \pi_{\text{cyclohexyl}} = 3.30 - 2.51 = 0.79.$$

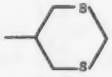
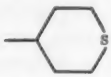
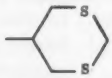
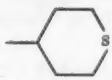
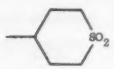
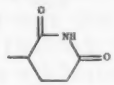
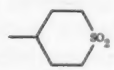
Adding this value of 0.79 to 2.83 for the cyclohexylnitrosourea derivative yields a log *P* value of 3.62 for compound 2 (2-adamantyl) of table 1. The measured value for the log *P* of the 1-adamantyl isomer is 3.56. This good agreement again illustrates the advantages to be gained in calculating log *P* values for new drugs before synthesis. For compound 23 of table 1, the experimental value of 1.53 for log *P* of the analogous chlorine derivative was used as a reference: 1.2, which is twice the difference between π values for chlorine and fluorine, was subtracted from this.

Log *P* values for these nitrosoureas are quite difficult to measure because of hydrolysis; however, the internal self-consistency between the measured values in table 1 leads us to believe that these values are sufficiently accurate for our purposes. For example, the measured log *P* value for compound 10 (*R* = *trans*-4-methylcyclohexyl) is 3.30. Its calculated value (calculated from the cyclohexyl derivative) is 2.83 + 0.50 = 3.33. The measured value for compound 22 is 0.57. It can be calculated from compound 15 as follows:

$$\text{Log } P_{ClCH_2CH_2N(NO)C(=O)NHCH_2CH_2Cl} - \pi_{CH_2CH_2} - \pi_{Cl} = 1.53 - 1.00 - 0.39 = 0.14.$$

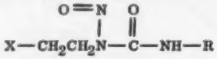
TABLE 1.—Antileukemic activity of nitrosourea derivatives



Compound No.	X=	R=	NSC No.	Log P	Log $\frac{1}{C}$ Observed	Log $\frac{1}{C}$ Calculated*	Δ Log $\frac{1}{C}$
1	Cl	4-*t*-Butylcyclohexyl	95439	4.51	3.03	3.061	0.03
2	Cl	2-Adamantyl	129968	3.62	3.24	3.533	0.29
3	Cl	*cis*-2-Chlorocyclohexyl	84954	2.73†	3.73	3.916	0.19
4	F	4-Methylcyclohexyl	125649	2.70	3.78	3.927	0.15
5	Cl	3-Methylcyclohexyl	93170	3.30	3.79	3.681	0.11
6	Cl	Norbornyl	88106	2.98†	3.91	3.817	0.09
7	Cl	Cyclopentyl	90330	2.19†	3.94	4.104	0.16
8	F		132085	1.54†	3.94‡	4.286	0.35
9	Cl	Cyclohexyl	79037	2.83†	3.97	3.877	0.09
10	Cl	*trans*-4-Methylcyclohexyl	95441	3.30†	4.00	3.681	0.32
11	F	Cyclohexyl	87974	2.23	4.01	4.091	0.08
12	Cl	*trans*-2-Chlorocyclohexyl	88104	2.73	4.03	3.916	0.11
13	F	Norbornyl	93492	2.38	4.06	4.041	0.02
14	Cl		103534	2.07†	4.06	4.141	0.08
15	Cl	CH_2CH_2Cl	409962	1.53†	4.07	4.288	0.22
16	Cl		128303	2.08†	4.20	4.138	0.06
17	F		114459	1.43†	4.23	4.312	0.08
18	Cl	4-Acetyloxycyclohexyl	117624	2.56	4.23	3.978	0.25
19	F		106767	−0.41	4.43	4.546	0.12
20	Cl		95466	0.37†	4.52§	4.494	0.03
21	Cl		105763	0.19†	4.55	4.512	0.04
22	Cl	H	47547	0.57†	4.58	4.469	0.11
23	F	CH_2CH_2F	91728	0.33	4.66	4.498	0.16

*Calculated using equation 2.
†Experimentally determined values.
‡This data point not used in the derivation of equation 2.
§This molecule is about 20%–25% ionized at physiologic pH.

TABLE 2.—LD10s of nitrosoureas in mice



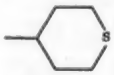
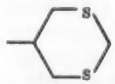
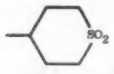
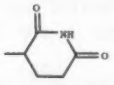
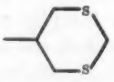
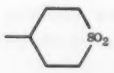
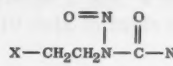
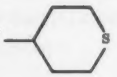
Compound No.	X=	R=	NSC No.	Log P	Log $\frac{1}{C}$ Observed	Log $\frac{1}{C}$ Calculated*	Δ Log $\frac{1}{C}$
1	Cl	4-*t*-Butylcyclohexyl	95439	4.51	2.76	2.943	0.18
2	Cl	3-Methylcyclohexyl	93170	3.30	3.24	3.509	0.27
3	Cl	*cis*-2-Chlorocyclohexyl	84954	2.73	3.30‡	3.552	0.25
4	Cl	Cyclopentyl	90330	2.19†	3.34	3.875	0.53
5	F	4-Isopropylcyclohexyl	132086	3.53	3.39	3.427	0.04
6	Cl	4-Ethylcyclohexyl	129967	3.83	3.42	3.293	0.13
7	F	4-Ethylcyclohexyl	129966	3.23	3.51	3.549	0.04
8	F	Norbornyl	93492	2.38	3.55	3.826	0.28
9	Cl	Norbornyl	88106	2.98†	3.58	3.641	0.06
10	Cl	Cyclohexyl	79037	2.83†	3.61	3.692	0.08
11	F	4,4-Dimethylcyclohexyl	132920	3.23	3.67	3.549	0.12
12	F	Cyclohexyl	87974	2.23	3.67	3.865	0.19
13	Cl	*trans*-2-Chlorocyclohexyl	88104	2.73†	3.69	3.552	0.14
14	Cl	CH_2CH_2Cl	409962	1.53†	3.72	4.005	0.28
15	F	4-Methylcyclohexyl	125649	2.70	3.72	3.724	0.00
16	F		114459	1.43†	3.76	4.019	0.26
17	F		132085	1.54†	3.79	4.003	0.21
18	Cl	*cis*-3-Ethoxycarbonylcyclohexyl	102724	3.06	3.79	3.612	0.18
19	Cl	*trans*-4-Ethoxycarbonylcyclohexyl	103548	3.06	3.84	3.612	0.23
20	Cl	*trans*-4-Methylcyclohexyl	95441	3.30†	3.84	3.509	0.33
21	Cl	2-Cyclohexenyl	132921	2.45†	3.87	3.785	0.09
22	F	4-Acetyloxycyclohexyl	121248	1.96	3.88	3.927	0.05
23	Cl	4-Acetyloxycyclohexyl	117624	2.56	3.91	3.776	0.13
24	F		106767	−0.41	3.92	4.039	0.12
25	Cl		95466	0.37†	4.06	4.088	0.03
26	Cl		128303	2.08†	4.09	3.901	0.19
27	Cl		105763	0.19†	4.22	4.084	0.14

TABLE 2.—LD10s of nitrosoureas in mice—Continued



Compound No.	X=	R=	NSC No.	Log P	Log 1/C Observed	Log 1/C Calculated*	Δ Log 1/C
28	Cl		103534	2.07†	4.26	3.903	0.36
29	F	CH_2CH_2F	91728	0.33	4.41	4.087	0.32

*Calculated using equation 4.
†Experimentally determined values.
‡These values not used in the derivation of equation 4.

While one generally finds better agreement between observed and calculated log P values, the inaccuracy in the present instance is very likely to be less than that of the biologic activity (log $1/C$).

RESULTS

Table 1 shows the lipophilic character and the antileukemic activity of 23 nitrosoureas, each having the same general structure but with considerable variation in the R position. X is either chlorine or fluorine. These compounds were tested in BDF_1 mice inoculated intracerebrally (ic) with 10^4 cells of L1210 leukemia; the drugs were administered by intraperitoneal (ip) injection. In equations 1 and 2, C refers to the molar concentration of drug required to give a T/C of 175%:

	n	r	s	
$\log \frac{1}{C} = -0.282(\pm 0.07)\log P + 4.639(\pm 0.17)$	22	0.886	0.189	(1)
$\log \frac{1}{C} = -0.0568(\pm 0.042)(\log P)^2 - 0.0689$ $(\pm 0.17)\log P + 4.527(\pm 0.17)$	22	0.922	0.163	(2)

T/C refers to the mean survival time of the treated animals (T) over that of the control animals (C) × 100. Thus, a T/C of 175% indicates a 75% increase in lifespan of the treated animals beyond that of control animals. For a mathematical correlation, it is of the utmost importance to have strictly comparable data where a *standard* response is related to molar concentration. The standard response of T/C of 175% was selected for comparison purposes. T/C responses were plotted,[5] a best fit curve was drawn to these responses, and the dose was determined based upon the selected standard T/C value.

Equations 1 and 2 were derived from the data in table 1. In these equations, C is the molar concentration producing a T/C of 175%, P is the octanol-water partition coefficient, r is the correlation coefficient, s is the standard deviation, and the figures in parentheses are the 95% confidence intervals on parameters. The number of data points used in the formulation of the equations is represented by n (in this case, 22). The data point for compound 8 was not used in the curve fitting.

Equation 2 is a statistically significant improvement over equation 1 ($F_{1,19} = 8.06$; $F_{1,19\ \alpha\ 0.01} = 8.2$). Determination of how valid this second-order equation is must await further testing; this is more easily seen in figure 2 where the experimental data are plotted against log P. The line is the theoretical line given by equation 2. Since so few data points with log P values of zero or less have been tested, it is hazardous to say at this time that activity will fall off with negative log P values. It is possible that the "linear portion" of the curve extends further and the testing of drugs that are less lipophilic would yield drugs with larger log $1/C$ values.

Unfortunately, the variation in R is such that we do not have suitable electronic or steric parameters to assess the structure-activity role of these variables. The only one of the three major physiocochemical parameters (4,5) (electronic, steric, or hydrophobic) that we can study in mathematical terms is the hydrophobic parameter. This characteristic of drugs is operationally defined by log P where P is the

[5] Data extracted from the screening data summaries of Drug Research and Development, DCT, NCI Contract Screening Program.

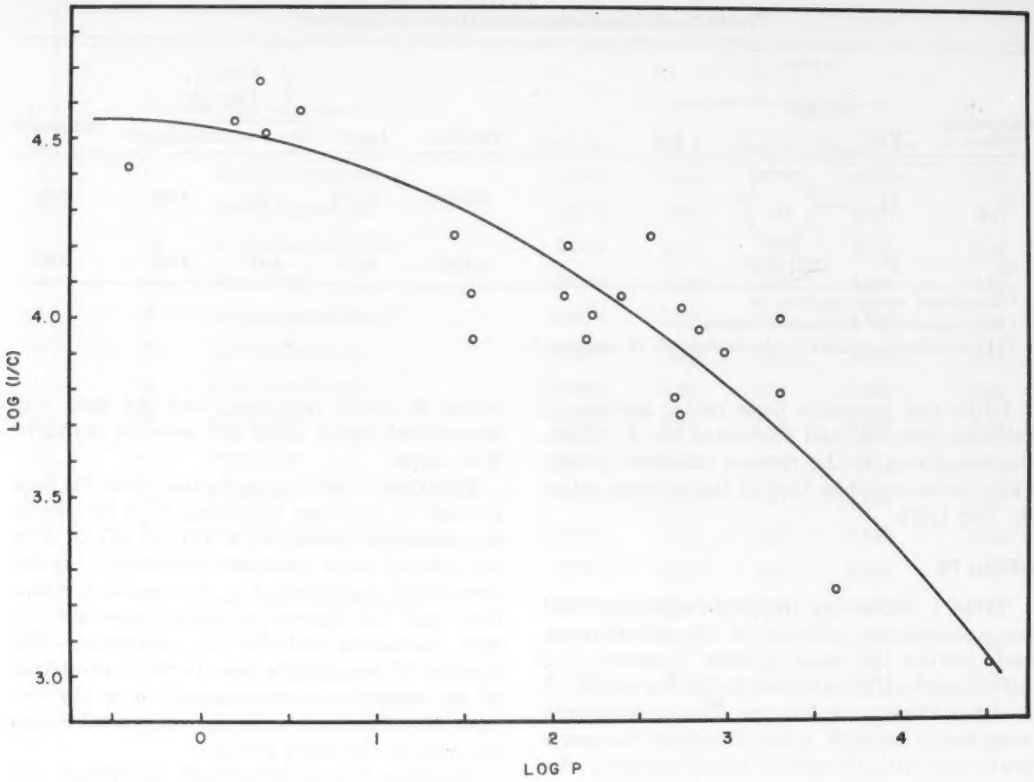


FIGURE 2.—Experimental data (log $1/C$) for 23 nitrosourea derivatives plotted against log P.

octanol-water partition coefficient. Considering the simplicity of the model, equation 1 gives a good correlation. Not only are we neglecting specific steric and electronic effects in this model, we have not included a term to take care of the variation in the chemical reactivity of X. We did explore this possibility by adding a dummy parameter to both equations 1 and 2 (molecules having a chlorine molecule were assigned a value of 1, and those with a fluorine molecule were assigned a value of 0). This did not result in improved correlation. The difference in activity of these two types of molecules appears to be related to their differences in lipophilic character. However, before ruling out the value of the difference in chemical reactivity of fluorine and chlorine, a better selection of derivatives should be studied. At present, it is clear that this is not a structural feature of major importance.

The negative slope of equation 1 indicates that increasing the lipophilicity of the nitrosoureas yields compounds less active against ic implanted L1210 leukemia in mice. This is clearly evident in figure 2. From our own experience in comparing activity with log P values, this is a rather unusual structure-activity relationship. Of over 1000 such equations from all kinds of systems now in hand we have found fewer than two dozen where essentially all of the derivatives tested fall on the right-hand side of a parabola correlating log $1/C$ and log P.

Taking the derivative (d log $1/C/d$ log P), setting this equal to zero, and solving for P, yields the parameter we have called log P_0 (6). This value, calculated from equation 2, is –0.6 (range, –7.6 to 0.5). The confidence intervals on this parameter are not centered (7).

Building potency into the nitrosoureas, of course, is only half the problem of drug devel-

opment; the other half is minimizing toxic side effects. Equations 3 and 4 have been derived from the LD10 data in table 2:

$$\log \frac{1}{C} = 0.210(\pm 0.08)\log P + 4.232(\pm 0.21) \quad n = 28,\ r = 0.737,\ s = 0.232 \quad (3)$$

$$\log \frac{1}{C} = -0.0688(\pm 0.050)(\log P)^2 + 0.0593(\pm 0.21)\log P + 4.066(\pm 0.21) \quad n = 28,\ r = 0.809,\ s = 0.206 \quad (4)$$

These equations correlate structure with the very crude LD10 measure of toxicity. Equations 3 and 4 are similar to equations 1 and 2; however, there is a difference in log P_0. The value calculated from equation 4 is 0.43 (range, –3.7 to 1.18). Although the confidence interval on this parameter is not tight, it is better than in the case of equation 2. Log P_0 for toxicity is about 1 log P unit higher than for potency. This indicates that not only should one be able to produce more potent drugs by making less lipophilic compounds, but that such compounds should also be less toxic (at least insofar as LD10 is a suitable measure of toxicity).

In a different type of structure acting against L1210 leukemia in mice, a different dependence of activity on lipophilic character is found. The imidazole carboxamides (fig 3) are unusual in that they are effective against both L1210 leukemia and melanoma (1, p 754). These compounds were tested in BDF_1 mice inoculated ip with 10^5 L1210 leukemia cells; the compounds were also given ip. The standard response selected was a T/C value of 150%. A dose-response curve was plotted [6] and the dose corresponding to the standard response was determined.

Equations 5 and 6 have been derived from the data in table 3.

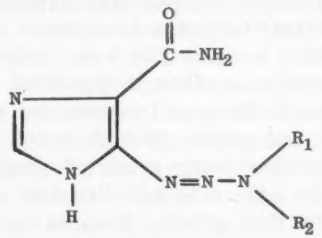


FIGURE 3.—Parent structure of the imidazole carboxamides.

[6] *See* footnote 5.

$$\log \frac{1}{C} = 0.299(\pm 0.18)\log P + 3.296(\pm 0.21) \quad n = 10,\ r = 0.800,\ s = 0.212 \quad (5)$$

$$\log \frac{1}{C} = -0.276(\pm 0.19)(\log P)^2 + 0.586(\pm 0.24)\log P + 3.454(\pm 0.18) \quad n = 10,\ r = 0.929,\ s = 0.146 \quad (6)$$

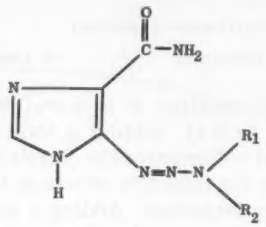
Log P_0 from equation 6 is found to be 1.1 (range, 0.77 to 2.4). Adding a term in σ^* to equation 6 did not improve the correlation. This indicates that the inductive effects of R_1 and R_2 are not highly important. Adding a term in E_s (8), Taft's steric parameter, to equation 6 (but not to equation 5) yields a significant improvement as shown in equation 7. Comparing equations 6 and 7 via the F test we find: $F_{1,5} = 6.7$; $F_{1,5\ \alpha\ 0.05} = 6.6$.

$$\log \frac{1}{C} = -0.168(\pm 0.15)E_s + 0.539(\pm 0.18)\log P - 0.280(\pm 0.14)(\log P)^2 + 3.430(\pm 0.14) \quad n = 10,\ r = 0.969,\ s = 0.104 \quad (7)$$

Log P_0 calculated from equation 7 is 0.96 (range, 0.75 to 1.5) which is close to that value calculated using equation 6. Although equation 7 is statistically more significant than equation 6, it must be viewed with considerable reservation since equation 7 has a large number of terms for only ten data points; also, to introduce E_s it was necessary to sum the values for R_1 and R_2. Another reason for suspecting the improvement obtained with the E_s term is that much of the improvement in correlation comes from a better fit of the unique derivative where R_1 = hydrogen and R_2 = ethyl. This is the only derivative where R_1 = hydrogen. The negative coefficient with the E_s term means that large groups promote activity (the larger the group, the more negative its E_s value; *see* table 3). This indicates that such sterically demanding groups as *sec*-butyl and *iso*-butyl do not hinder antileukemic activity; in fact, they appear to promote it slightly.

It is most significant that one of the two R groups in the parent molecule for the imidazole carboxamides (fig 3) must be the size of CH_3 or smaller (the compounds shown in table 4 have larger groups and are completely inactive). Compound 11 of table 3 is an exception to this general rule; R_1 and R_2 are both large groups and yet this is the most active drug in table 3. Compound 11 is poorly predicted by equation 6. It seems most likely that this drug is operating via a quite different mechanism; in fact, it could possibly function like the alkylating

TABLE 3.—Antileukemic activity of imidazole carboxamide derivatives



Compound No.	R_1=	R_2=	NSC No.	Log P	ΣE_s	Log $\frac{1}{C}$ Observed	Log $\frac{1}{C}$ Calculated*	Δ Log $\frac{1}{C}$
1	H	C_2H_5	404275	−0.39	1.17	2.96	3.184	0.22
2	CH_3	CH_2CH_2OH	83112	−0.79	−0.77	2.99	2.819	0.17
3	CH_3	CH_3	45388	−0.24†	0.00	3.26	3.298	0.04
4	CH_3	C_5H_{11}	87981	1.76	−0.40	3.44	3.632	0.19
5	CH_3	Cyclohexyl	83111	1.77	−0.79	3.70	3.628	0.07
6	CH_3	$CH_2C_6H_5$	83695	1.54†	−0.38	3.70	3.703	0.00
7	CH_3	$CH_2CH(CH_3)_2$	83113	1.06	−0.93	3.75	3.766	0.02
8	CH_3	$CH_2CH_2CH_3$	76418	0.79	−0.36	3.75	3.741	0.01
9	CH_3	$CH(CH_3)CH_2CH_3$	144216	1.08†	−1.13	3.84	3.766	0.07
10	CH_3	$(CH_2)_3CH_3$	70874	1.26	−0.39	3.90	3.755	0.14
11	CH_2CH_2Cl	CH_2CH_2Cl	82196	1.54	−1.80	4.33‡	3.703	0.63

*Calculated using equation 6.
†Experimentally determined values.
‡This data point not used in the derivation of equations 5–7.

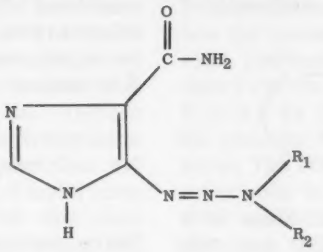
agents of table 1. Since the steric and electronic demands of R in the molecules of table 1 appear to be slight, and since the electron density on the nitrogen atoms of the nitrosoureas is low because of the attached carbonyl group (making them comparable to the nitrogens of the imidazoles), it was concluded that it might not be totally out of place to consider compound 11 as acting similarly to the nitrosoureas. Accordingly, if we introduce its log P value of 1.54 into equation 2, we obtain a calculated value of log $1/C$ of 4.50 which is close to the observed value of 4.33. Of course, different T/C definitions are involved, so that comparison is not strictly proper; nevertheless, this does suggest that further investigation of this point would be worthwhile.

DISCUSSION

Possibly the first thing which should be said about the results in table 1 is that the molecules which are well predicted are the least interesting. Their structure-activity relationship is contained succinctly in the few characters of equation 2. It is the poorly fit molecules that are most interesting. Through the study of these exceptions further insight into the mode of action of the nitrosoureas may be gained.

The adamantyl derivative in table 1 (compound 2) is less inactive than expected; this suggests that there may be a steric effect of R but that it is not very serious. Compound 10 is about twice as active as predicted. Its exceptional activity must be associated with its geometry, but exactly how, it is not possible to say. Another poorly predicted compound is No. 18, the ester. The fact that this agent is more active than expected from its log P value may be due to a change in log P resulting from hydrolysis. The π value for CH_3COO^- is −0.27, while that for the OH which would result via hydrolysis is −1.16. If we assume that hydroly-

TABLE 4.—Inactive imidazole carboxamide derivatives



Compound No.	R_1 =	R_2 =	NSC No.
1	Ethyl	Ethyl	52371
2	Butyl	Butyl	45396
3	Octyl	Octyl	404182
4	Benzyl	Benzyl	52372
5	CH_2CH_2CN	CH_2CH_2CN	92157
6	$CH_2CH_2OCH_3$	$CH_2CH_2OCH_3$	143689
7	CH_2CH_2OH	CH_2CH_2OH	144215
8	CH_2CH_2OH	Benzyl	144217
9	$CH_2CH_2OCH_3$	$C(CH_3)_3$	146038
10	$CH_2CH_2CH_2CH_2CH_2$		406800

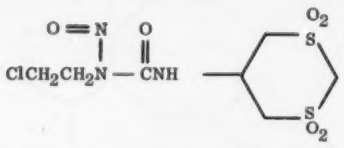
sis occurs rapidly, then a log P value of 1.67 is more appropriate. Substituting this figure in equation 2 yields a calculated value of 4.26 which is closer to the experimental value of 4.23. The most poorly fit compound in table 1 is No. 8, which was not included in the derivation of equations 1 and 2. It is less active than expected and, in view of the good fit of compound 16, one is at a loss to explain its low activity.

Compound 20 of table 1 is well predicted; however, it must be pointed out that this substance which has a pK_a value of 7.92 [7] would be about 20%–25% ionized at physiologic pH. We have not applied this small correction to the log 1/C value for this substance.

One compound not included in table 1 is the congener shown in figure 4. This compound shows only marginal activity (T/C=138% at a dose of 37 mg/kg) despite the fact that its log P value is −0.9. Neither equation 2 nor figure 2 would suggest this result. One must consider which of the important properties of this moiety (electronic, steric, or hydrophobic) is responsible for this lack of activity. As mentioned previously, equation 2 would indicate that this compound should not be so weakly active on the basis of its log P value alone. When groups as bulky as adamantyl, t-butylcyclohexyl, or norbornyl are active and reasonably well predicted by equation 2, it seems unlikely that the steric demands of the dithian dioxide function could be responsible for the low activity of this compound. This leaves us with the electronic character of the disulfoxide functions as the culprit. Since these functions are two carbon atoms removed from the urea moiety, their electronic effect on this function would be considerably dampened. Although it may be on this amino nitrogen that one should look for the cause of the low activity, it seems more likely that the activation of a methylene group flanked by the two sulfoxide functions is responsible for the unde-

[7] W. J. Haggerty. Personal communication, Midwest Research Institute.

sirable effect. Price and Oae (9) have discussed the high acidity of hydrogens on such functions. The pK_a value in 50% methanol of



has been found to be 8.18±0.23.[7] Since it is well known (10) that 50% alcohol may suppress the ionization of an acid by as much as 0.5 to 1.0 pK_a unit, this nitrosourea could be 50% or more ionized at physiologic pH. This alone would not account for the low activity. If only 50% of the drug were available, this would only change the log $1/C$ value by 0.3. However, the carbanion form of the dioxide might be readily oxidized in the mouse, or the carbanion might contribute to the instability of the drug in some other way.

It is clear from table 1 and figure 2 that presently we do not have a good distribution of nitrosoureas from the point of view of log P. Most of the compounds have log P values in the range of from 1.5 to 3.0. It would be desirable to test a few more with high log P values (3.5 to 4.5) to be surer of the apparent inactivity of drugs having high lipophilic character. Five drugs with log P values near zero are all highly active, but this region needs further exploration. The real unknown is the region beyond log P of zero and it is the exploration of this region which holds most promise. It may be that the curve in figure 2 is "linear" over a greater range of log P values than seems to be the case at present. Most important is the fact that toxicity as measured by LD10 seems to peak at a higher log P value than potency. This point can be tested by checking more hydrophilic drugs both for antileukemic activity and toxicity. Equations 2 and 4 can be developed to maximize the therapeutic index.

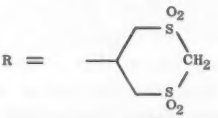
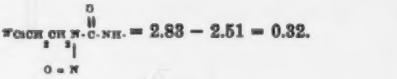


FIGURE 4.—Congener not included in table 1 as a substituent for the R position.

To obtain derivatives with log P values less than zero, what kind of functions should be considered? Taking the derivative where X = chlorine and R = cyclohexyl as a reference point, we can subtract $\pi_{cyclohexyl}$ from this log P to obtain:

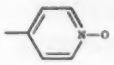
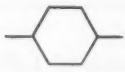
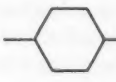
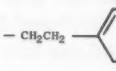
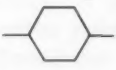


Hence, we must look for R functions having π values of −0.3 or less.

Table 5 lists some possibilities. These values have been *estimated* from aliphatic compounds (3). Many others could be designed.

Since there is considerable evidence (11) that nitrosoureas are acting as alkylating agents, one might expect to find other highly

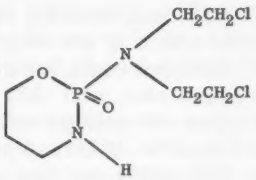
TABLE 5.—Estimated π values for various R functions

Function	Estimated π values
	−1.69
	−2.24
	−1.74
	−0.93
$CH_3CON(CH_3)_2$	−0.77
	−0.69
	−0.43
$CH_3CH_2CON(CH_3)_2$	−0.37
$CH_3CH_2SO_2CH_3$	−0.26
CH_3CH_2CN	0.16

effective alkylating agents which have quite different structures with low log P values. As yet, not many partition coefficients have been determined for these compounds. The three examples shown in figure 5 (in addition to compound 11 of table 3) serve to illustrate this point. These examples suggest that further studies of the relationship of antineoplastic activity and log P should be undertaken. Of course, one must not let equations 2 and 4 completely dominate drug design with this class of compounds. Because of the limitations imposed by the data in hand, no estimate of the importance of the stereoelectronic features of the nitrosourea molecule (which make this such a good system for antileukemic activity) can be made. Until more is known, we cannot skillfully manipulate these parameters to maximize potency and minimize toxicity.

Since the leukemic cells are being introduced into the mouse brain, to be effective the drugs must penetrate into this region. It has been shown (7) that nonspecific hypnotics have a log P_0 of 2.0. In light of this fact one wonders why the ideal log P for the nitrosoureas is so much lower. The reason for this may be that if these compounds tend to bind to serum proteins or other macromolecules, alkylation will occur and the drug will be stopped from reaching its active site. It is known that a wide variety of neutral compounds bind to serum protein in direct proportion to their log P values (12).

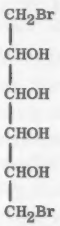
Turning to the imidazole carboxamides of table 3, we find that drugs with log P values



Cyclophosphamide

NSC-26271

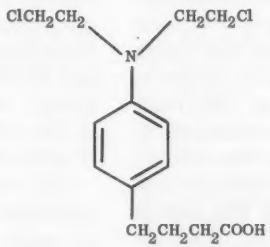
log P = 0.63



Dibromomannitol

NSC-94100

log P = −0.24



Chlorambucil

NSC-3088

Estimated log P = 0.30

FIGURE 5.—Structures of three alkylating agents and their log P values.

well-spread on both sides of log P_0 are represented in the set. Abraham et al (13) have suggested that this class of compounds is active because it resembles guanine and may therefore interfere with its incorporation into DNA or RNA. If this is true, one might expect equation 6 to predict a log P_0 near log P for guanine. While log P for guanine has not been measured in the octanol-water system, it has been measured in the butanol-water system. Thus, its log P in octanol-water can be calculated (3). The calculated value is -1.00 which is quite far from the log P_0 value of 1.1 calculated using equation 6. This suggests some other mechanism of action for the imidazole carboxamides.

The complete inactivity of compounds with R_1 and R_2 = ethyl and other compounds of this type shown in table 4 is a point which needs further study.

Cancer chemotherapy is one of the most challenging and difficult problems the medicinal chemist faces. It is generally conceded that a truly effective drug must destroy every malignant cell in the host. When rapidly metastasizing cancer cells are being distributed to many parts of the body, it is highly likely that malignant cells will be deposited in both fatty and aqueous regions, eg, the central nervous system as well as the blood system. An effective drug must then be able to penetrate both aqueous and lipophilic phases of the body and attain high enough concentrations in these areas to destroy all of the neoplastic cells. An inspection of drugs currently found to be effective against L1210 leukemia reveals that a very large majority are compounds with low log P values (approximately zero or less). While such drugs may have striking results against leukemic cells in the hydrophilic phases of the body (eg, the blood), they no doubt penetrate poorly into highly lipophilic areas (eg, the central nervous system). From this it follows that in contrast to bacterial infections, one should not focus too heavily on log P_0 alone in cancer chemotherapy. Although a single drug having log P_0 might produce a high T/C value, this may be a transitory effect and malignant cells residing in areas poorly penetrated by compounds of log P_0 may eventually attain a growth rate which ultimately destroys the host. It might be wiser to use two drugs, one which is about 1 log unit below log P_0 and the other which is about 1 log unit above log P_0. In this way the most important area around log P_0 would be well covered and, in addition, the extreme hydrophilic and hydrophobic regions would also be penetrated.

For example, it has been pointed out recently that NSC-45388 (compound 3, table 3), while being effective against melanoma, does not penetrate the central nervous system well and that metastases develop in this area while the disease outside the central nervous system is controlled (14). Since considerable evidence has accumulated (7,15) indicating that log P_0 for passive penetration of the central nervous system is about 2.0, it is not surprising that NSC-45388 which has a log P value of -0.24 does not penetrate well. It would be most interesting to use a combination of two of the imidazoles from table 3, for example, NSC-45388 and, possibly, compound 10 (NSC-70874), which is not only more active than NSC-45388 but which has a log P value slightly above optimal.

Another interesting candidate would be compound 5 (NSC-83111), which is more active than NSC-45388 and has a near optimal log P value for penetration of the lipophilic central nervous system.

Important progress against leukemia has been made in recent years using a combination of drugs selected to strike at malignant cells during various phases of mitosis. A well-planned study of 12 drugs acting in various ways at the molecular level has been reported by Skipper et al (16). Although log P values are not available for most of the drugs used in this thoughtful study, probably only two (BCNU and CCNU) have log P values greater than 1. Rough calculations would indicate that most of the others have log P values of approximately zero or less.

Our results indicate that in sophisticated combination chemotherapy where several drugs are used to attack different molecular processes in malignant cells, more effective control of cancer is likely to be achieved by using a lipophilic and a hydrophilic congener for each process under attack.

The results summarized by equations 2 and 6 constitute powerful means for help in the decision-making process which was raised in

the discussion of the structure shown in figure 1. Which of the millions of possible derivatives of this compound should be made and tested? Although figure 2 should be "fleshed out" using compounds of high lipophilicity, it seems quite unlikely that molecules having log $1/C$ of 4.5 or greater will be found among compounds with log P values greater than 1.5. Since log P values can be accurately estimated without synthesizing a compound (3), random synthesis can be minimized. At this stage, molecules should be made so that redundancy in log P is avoided. The most likely area of investigation is among compounds with log P values between -1 and $+1$. The infinity of compounds outside this region can be neglected for rational reasons.

In a recent study of dinitrophenylaziridine carboxamides (fig 6), Khan and Ross (17) found that π_R plotted against log $1/C_{ED90}$ produced a good linear relationship with a negative slope; that is, the more hydrophilic the drug, the more activity it showed against Walker carcinosarcoma 256. They used π constants from the ether-water system; however, since these constants are known to be rather well correlated with octanol-water partition coefficients (3), their results are directly comparable to ours. Their most hydrophilic derivative was that in which R was hydrogen. We have found this compound to have a log P value of -0.02 in the octanol-water system. Hence, their results for this class of alkylating agents indicate that one should look for more active derivatives among compounds having negative log P values.

Still another set of experimental data related to the present study is that obtained by

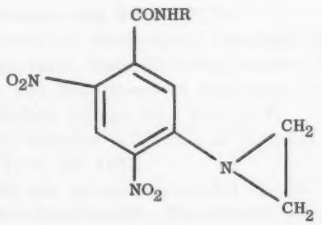


FIGURE 6.—Parent structure of the dinitrophenylaziridine carboxamides.

Hudson et al (18) in the treatment of Walker carcinosarcoma 256 using esters of the type:

$$CH_3SO_2O(CH_2)_nOSO_2CH_3$$

A plot of their data showed that there was a parabolic relationship between relative activity and the ether-water partition coefficients. Peak activity was found in the derivative where n was 4. Log P for their ether-water partition coefficient was -0.74. The log P value for busulfan (NSC-750; Myleran) (n = 4) in the octanol-water system is -0.47.[8] This agrees quite well with the optimum log P of -0.6 found for the nitrosoureas and suggests a similarity in mechanism of action.

Our work, together with that of others, provides evidence that substituent constants and regression analysis can be helpful in the decision-making processes involved in the design of anticancer drugs.

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Effect of 1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU; NSC-79037) in Sesame Oil Administered Intramuscularly in the Treatment of Experimental Brain Tumors [1,2,3]

William R. Shapiro [4,5,6,7]

SUMMARY

A parenteral preparation of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) dissolved in sesame oil was administered intramuscularly (im) to C57BL/6J male mice bearing intracerebrally implanted Glioma 261, Glioma 26, or Ependymoblastoma A (EpA). CCNU was given on Day 2, Day 7, or Day 14 in individual experiments in doses at or below the LD10. The drug-sesame oil preparation was highly efficacious in prolonging the lifespan of the animals treated on all 3 days; the maximum increase in median lifespan exceeded 400% in mice carrying Glioma 261, 385% in mice carrying Glioma 26, and 476% in mice carrying EpA. Long term survival was 50% in almost all experiments. Sesame oil alone was nonlethal and histologic examination revealed that both oil and CCNU plus oil produced only minimal muscle changes. The CCNU-in-sesame oil preparation for im administration is easily formulated and perhaps could be well-tolerated when administered parenterally to patients with cancer.
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[2] CCNU was supplied by Drug Research and Development, Chemotherapy, National Cancer Institute (NCI).

[3] Supported in part by grant CA-08748 from NCI, National Institutes of Health (NIH), and grant T-537 from the American Cancer Society.

[4] Recipient of Research Career Development Award 5-K4-NS-50,294 from the National Institute of Neurological Diseases and Stroke, NIH.

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Previous studies have demonstrated the effectiveness of a number of chemotherapeutic agents against an intracerebrally (ic) implanted murine glioma (1–3). Among the agents tested, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) proved to be one of the most effective (3). The drug, one of the group of nitrosoureas, is lipid-soluble and has been shown to cross the blood-brain barrier to enter the brain, brain tumors, and cerebrospinal fluid (CSF). The drug is so insoluble in aqueous media that in the initial experiments in animals the dry powder was suspended in a steroid suspending vehicle and injected intraperitoneally (ip). For clinical use, CCNU is supplied in capsule form for oral administration; no parenteral preparation is yet available. Studies on the distribution of CCNU in ani-

mals (9) and man (10) suggest that when CCNU is administered by the oral route, it is broken down before absorption takes place, although both toxicity and clinical efficacy have been demonstrated. Because absorption of the orally administered drug is variable and because identification of the parent compound in body tissues after administration is difficult, an effort was made to establish a parenteral preparation of CCNU. It was found that the drug dissolves readily in sesame oil. In this vehicle the drug was administered intramuscularly (im) to mice bearing intracranial brain tumors, and the effectiveness of this combination was tested. The results of these studies are reported in this paper.

MATERIALS AND METHODS

Details of the model methodology have been reported (1,2). C57BL/6J male mice weighing 20–24 g were used. Three of the four murine gliomas initially used in the model were utilized for these studies. Glioma 261 (induced by Seligman and Shear [4]) and Glioma 26 (induced originally by Sugiura [5]) were both obtained from Microbiological Associates, Inc., Bethesda, Md. Ependymoblastoma A (EpA) is a mutant subline of the original murine ependymoblastoma induced by Zimmerman and Arnold (6).

The tumors were carried by subcutaneous (sc) transplantation. For ic implantation, sc tumors 12–14 days old were cut into 1-mm fragments and the fragments were mounted in the tip of a spinal needle to which a metal guard had been fitted. The tip of the needle was inserted to a constant depth through the scalp and skull of mice under light ether anesthesia. The tumor fragment was extruded with the use of the needle's obturator, and implanted in the right cerebral hemisphere about 2 mm below the surface of the brain. In this manner, tumors could be implanted in 75–100 mice in 1.5 hours. Ninety-eight percent of these inoculated animals died of their brain tumors by Day 60 at intervals varying with each of the tumors. The approximate median survival time (MST) of untreated animals bearing each of the tumors was as follows: Glioma 261, 25–27 days; Glioma 26, 26–30 days; EpA, 21–23 days (1).

In each chemotherapy experiment, 25 untreated mice served as tumor controls. Tumor-bearing animals receiving CCNU were divided into groups of ten animals for each dose level, and these groups were matched with groups of ten non-tumor-bearing animals (drug controls) which were similarly treated. Animals were observed after drug injection and the day of death was recorded for each mouse.

The results were analyzed as follows: the Wilcoxon rank sum test (7) permitted statistical comparison of the differences in survival time between the control and treated groups. Comparison of the MST of the treated animals (T) with that of the control animals (C) yielded the degree of increase in lifespan. Finally, the percentage of long term survivors (animals living longer than 60 days) was recorded.

The sesame oil was laboratory grade; it was sterilized (by autoclave) before being mixed with CCNU. The CCNU was weighed and dissolved in sesame oil to yield a dose for combined drug and oil of 0.005 ml/g of mouse body weight. The drug was injected into the mid-posterior thigh muscles of the right lower extremity of the mouse. The LD50 and LD10 were calculated by the method of Litchfield and Wilcoxon (8). The LD50 exceeded the highest dose used (70 mg/kg) and the LD10 for a single injection was approximately 60 mg/kg. Characteristic delayed toxicity was observed with non-tumor-bearing animals dying up to 68 days after receiving CCNU (9).

The effect of sesame oil alone was evaluated by determining (*a*) its chemotherapeutic efficacy, (*b*) its lethality, and (*c*) the effect of the injection itself on muscle. In a separate set of experiments, mice received im injections of sesame oil and sesame oil plus CCNU. One week later the animals were killed, the legs were amputated, and specimens of the treated muscle were prepared for histologic examination.

RESULTS

Chemotherapy Experiments

In each experiment, CCNU was given as a single im injection on 1 of the 3 days following tumor implantation, ie, on Day 2, Day 7,

or Day 14. Table 1 shows the effects of CCNU in sesame oil given to mice bearing ic implanted Glioma 261 tumors. Three experiments are represented. In the Day 2 experiment, five different doses of CCNU were used; in addition, sesame oil alone was given to another group of animals at a dose of 0.005 ml/g of body weight. Sesame oil alone was not lethal; however, it did not have any chemotherapeutic effect either. The table was constructed when an MST of 100 days was achieved. T/C values, therefore, exceeded 100/25 in each of the five dose groups with a median lifespan (expressed as percent of control) exceeding 400% in all cases. The increased survival was significant by Wilcoxon testing at all doses, and six to ten animals per dose survived longer than 60 days. Long term survivors usually remained alive for 6 months and were considered cured.

When the drug was administered on Day 7, only three doses were used. Again, noticeable increases in survival times were demonstrable, although somewhat fewer animals survived longer than 60 days. Almost identical results occurred when treatment was delayed to Day 14 in the third experiment shown in table 1.

Table 2 shows the results of CCNU injection in three separate experiments on 3 different days following implantation of Glioma 26 tumors. In the Day 2 experiment, additional groups were treated with 70-mg/kg doses of drug-oil preparation which had been allowed to remain standing in the open air for 60 and 90 minutes prior to administration. The overall results show a significant increase in survival times with all three doses tested, including the delayed 70-mg/kg doses. For the 90-minute delayed injection only seven animals survived longer than 60 days, while for all other doses at least nine animals survived. Although not statistically significant, the slightly reduced efficacy of the preparation may have been related to its prolonged exposure to the air.

When treatment was delayed until Day 7, significant increases in survival time again occurred in mice treated with doses of 50 and 70 mg/kg. The Wilcoxon test was bimodal at the 40-mg/kg level. Bimodality occurs when three of ten animals die at approximately the same time as the controls and the remaining animals survive longer than controls. Statistical analysis is not possible under such circumstances, although, in this case, the MST was still increased. There were fewer long term survivors in this experiment than in the early treatment experiment. When treatment was delayed to 14 days, the increase in survival time was not as great as that with earlier treatment and the number of long term survivors was also somewhat smaller.

TABLE 1.—Effect of CCNU in sesame oil administered im to mice bearing ic implanted G261 tumors

Day of treatment	Dose (mg/kg)	Median day of death (T/C)*	Significance (Wilcoxon test)	Median lifespan (% of control)	Animals surviving 60+ days/ total treated
2	20	>100/25	$P < .00001$	>400	6/10
	30	>100/25	$P < .00001$	>400	9/10
	40	>100/25	$P < .00001$	>400	10/10
	50	>100/25	$P < .00001$	>400	10/10
	70	>100/25	$P < .0001$	>400	8/10
	Oil	24.5/25	NS†	98	0/10
7	40	>100/27	$P < .001$	>370	5/10
	50	69/27	$P < .0001$	256	6/10
	70	>100/27	$P < .005$	>370	6/10
14	40	58.5/27	$P < .00001$	217	5/10
	50	57.5/27	$P < .001$	213	4/10
	70	>100/27	$P < .0001$	>370	7/10

*T/C: T = median day of death of treated animals; C = median day of death of control animals.

†NS = not significant.

TABLE 2.—Effect of CCNU in sesame oil administered im to mice bearing ic implanted G26 tumors

Day of treatment	Dose (mg/kg)	Median day of death (T/C)	Significance (Wilcoxon test)	Median lifespan (% of control)	Animals surviving 60+ days/ total treated
2	40	>100/30	$P < .00001$	>333	9/10
	50	>100/30	$P < .00001$	>333	10/10
	70	>100/30	$P < .00001$	>333	10/10
Delayed injection:					
60 min	70	>100/30	$P < .00001$	>333	9/10
90 min	70	>100/30	$P < .001$	>333	7/10
7	40	51.5/26	Bimodal*	198	4/10
	50	71/26	$P < .05$	273	6/10
	70	>100/26	$P < .01$	>385	6/10
14	40	59.5/28	$P < .0001$	213	5/10
	50	60/28	$P < .00001$	214	5/10
	70	57/28	$P < .005$	204	5/10

*Distribution includes early and late deaths; Wilcoxon analysis not possible. *See* text for details.

Experiments done with EpA are shown in table 3. Treatment on Day 2 produced significantly increased survival times at all three dose levels tested, with seven to nine animals surviving longer than 60 days. Treatment on Day 7 resulted in increases in the survival time for mice treated with the 40- and 50-mg/kg doses, but the 70-mg/kg dose proved to be toxic and the result was bimodal. Similarly, on Day 14, the 70-mg/kg dose was lethal, producing five early deaths. When treatment was delayed to Day 14 in EpA-bearing mice, the animals often already had symptoms of disease and were less able to tolerate the higher doses of the chemotherapeutic agent; lethality thus occurred at such delayed high doses.

Muscle Studies

Animals demonstrated no clinical toxic response to the combination of CCNU in oil other

TABLE 3.—Effect of CCNU in sesame oil administered im to mice bearing ic implanted EpA tumors

Day of treatment	Dose (mg/kg)	Median day of death (T/C)	Significance (Wilcoxon test)	Median lifespan (% of control)	Animals surviving 60+ days/ total treated
2	40	> 100/21	$P < .00001$	> 476	7/10
	50	> 100/21	$P < .00001$	> 476	9/10
	70	96.5/21	$P < .001$	460	7/10
7	40	63/23	$P < .005$	274	6/10
	50	> 100/23	$P < .01$	> 435	6/10
	70	34/23	Bimodal*	148	3/10
14	40	57/21	$P < .0001$	271	5/10
	50	50/21	$P < .005$	238	4/10
	70	20.5/21	Lethal	98	3/10

**See* footnote for table 2.

than that directly related to the chemotherapeutic agent. The coats of many animals became oily and shiny after the injection; this effect lasted several weeks. Animals surviving longer than 3 or 4 months frequently had some graying of the fur which persisted for the duration of their lives.

Preparations of muscle tissue from treated animals were compared to muscle from untreated control mice. The muscle from animals receiving sesame oil alone and that from mice receiving both CCNU and sesame oil was essentially identical. Most of the tissue appeared normal, but small foci of interstitial inflammatory cells were present in both samples. Cells were predominantly lymphocytic and mononuclear in nature, and tended to be located in the epimysial and perimysial connective tissue rather than in the endomysial tissue surrounding the individual muscle fibers. In each specimen there was a single small focus of several muscle fibers undergoing either necrosis, phagocytosis, or regenerative changes. The muscle tissue from the control animals showed no inflammatory or myopathic changes.

DISCUSSION

The purpose of this study was to test a parenteral preparation of CCNU. After a pilot study showed that sesame oil given im was not toxic to animals, the next step was to determine if the combination of the drug and the oil was efficacious. Previous studies had demonstrated the efficacy of CCNU suspended in a steroid suspending vehicle administered intraperitoneally (ip) to mice bearing ic implanted murine gliomas (3). A comparison of the present results with those reported previously follows.

With respect to Glioma 261, im administration on Day 2 produced results essentially identical to those obtained with ip administration on Day 2; similar results occurred with Day 7 ip and im administration (3). Similarly, there was very little difference with Day 14 administration, although there was some indication that the CCNU-in-oil preparation produced a few more long term survivors. For mice bearing Glioma 26, somewhat better results were obtained with the drug-oil preparation given im than with the same drug given ip (3). Median lifespans exceeding 333% occurred with all three doses given on Day 2 in the current experiment but on only one occasion with a dose of 70 mg/kg given ip (3). Similarly, while im treatment resulted in the cure of nine or ten animals (table 2), ip treatment at best produced three, four, or five long term survivors per group of ten mice. The previous studies with Glioma 26 did not include experiments on delayed therapy. With respect to EpA, there was no significant difference between ip and im injections on Day 2; experiments with delayed ip treatment had not been done before the present study. Thus, it would seem that im injections of CCNU dissolved in sesame oil can produce equal and perhaps better results than those obtained with ip treatment with CCNU suspended in the steriod suspending vehicle.

With respect to toxicity, the current studies suggest that the im route of administration does not produce significant systemic toxicity related to the oil per se. At the site of injection, although most of the muscle was entirely normal, several small foci of inflammatory cells were observed along with occasional myopathic changes. Such histologic lesions in animals receiving sesame oil alone and in those receiving the CCNU-in-oil preparation were identical.

Engel has demonstrated that muscle punctured by needles readily produces tracts of inflammatory cells and degenerating muscle fibers and that such changes occur even with electromyographic needles (27-gauge) and no injection of drug (11). It is likely that the changes seen in the muscles of mice in this study were secondary to the needle punctures alone.

Both peanut oil and sesame oil have been used as vehicles for depository administration of agents such as penicillin, pitressin, and epinephrine in man (12), permitting slow release and prolonged effect. It is possible that for some chemotherapeutic agents, including CCNU, prolonged absorption from the im depository site might produce increased effectiveness. Direct comparison of ip, im, and oral administration in the same test system would be required, however, to test this hypothesis.

It is also possible that sesame oil might alter the metabolism of CCNU by inhibiting liver microsomal enzymes. Fine and Molloy (13) have shown that sesoxane, a compound closely related to the sesame oil components sesamin and sesamolin, has such inhibitory activity. Examination of the hexobarbital sleeping time of mice given sesame oil, CCNU, and the combination of oil and drug would be of interest in this regard.

Distribution studies of CCNU have been carried out in animals (9) and in man (10). Rather rapid degradation of the parent compound was demonstrated, but radioactive label was detected in the plasma and CSF for prolonged periods irrespective of the site of the label. Although the drugs were active and toxic when given by the oral route, very little intact CCNU was identified in the blood of animals, and none in the blood of man after oral administration. In the mouse, the concentration of the parent compound in subcutaneous and ic murine gliomas as well as in brain tissue (14) was four times as high as that in the plasma. The difficulty in detecting the parent compound in the urine of animals and man suggests that degradation must occur either before absorption or coincident with absorption. Degradation to some compound other than the parent compound must occur in the gastrointestinal tract in man prior to absorption. The fact that the drugs are active and toxic when given by the oral route suggests that one of the degradation products must be active.

Clinical studies with orally administered CCNU have been undertaken by Hansen et al (15) and his associates (16) as well as here at the Memorial Sloan-Kettering Cancer Center. In general, in all of the studies CCNU produced the delayed toxicity usually seen with the nitrosoureas: depression in the counts of bone marrow constituents, platelets, white blood cells, and, to a lesser degree, red blood cells. Some clinical efficacy in patients with bronchogenic carcinoma and in several patients with brain tumor has been reported. Our own experience is similar, but we have found it difficult to predict the toxic response to a given dose of drug when it is administered orally. It is not known whether this difficulty relates to possible degradation of the compound, but such degradation is certainly a possibility. The parenteral preparation described in this study would probably be safe in man and could be made available for clinical use after additional toxicologic studies.

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Metabolism of 5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388) in Human and Animal Tumor Tissue [1,2]

N. S. Mizuno and E. W. Humphrey [3]

SUMMARY

In vitro experiments with Sarcoma 180 (S180) tumor slices showed that 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388) inhibited the uptake of radioactive precursors by RNA and DNA. The degree of inhibition was proportional to the concentration of the drug. NSC-45388 also inhibited the incorporation of RNA precursors by human tumor tissue in vitro. The rate of DNA synthesis was too low to allow assessment of the drug effect. The uptake of ^{14}C-labeled amino acids by protein fractions was less susceptible to the action of NSC-45388. The distribution of ^{14}C from methyl- and 2-labeled NSC-45388 in various tumor fractions (acid-soluble, lipid, nucleic acid, and protein) was similar in S180 and human melanoma tumors. With tumor microsomal preparations, ^{14}C-labeled HCHO was derived only from methyl-labeled NSC-45388, and ^{14}C-labeled 5-aminoimidazole-4-carboxamide was found only when 2-labeled NSC-45388 was used. When nucleic acids were present in the incubation mixture, they became labeled when the methyl-labeled compound was used. Radioactive 7-methylguanine was isolated from the nucleic acid hydrolysates. The alkylation of nucleic acids could not be demonstrated during photodecomposition of NSC-45388 to 5-diazoimidazole-4-carboxamide. The evidence indicated that in tumor tissue, methylation could take place via the 5-(3-monomethyl-1-triazeno)imidazole-4-carboxamide intermediate, which decomposed spontaneously to generate a methylating agent, according to a scheme which was proposed for normal tissue.

[Cancer Chemother Rep 56:465–472, 1972]

The antitumor drug, 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388), has been shown to possess a broad range of activity against rodent tumors (1) and some beneficial effects have been reported in some types of human tumors (2–6). In man and rats, the drug was shown to undergo demethylation and form formaldehyde and 5-aminoimidazole-4-carboxamide (NSC-7784; AIC) in vitro and in vivo (7–10). When methyl-labeled NSC-45388 was used, radioactive 7-methylguanine was isolated from nucleic acid fractions of rat tissues, indicating that NSC-45388 has properties of a potential alkylating agent. NSC-45388 can also undergo photodecomposition under the influence of long-wave ultraviolet light and can be converted to 5-diazoimidazole-4-carboxamide (NSC-22420; Diazo-ICA). Since NSC-22420 itself inhibits growth in bacterial cells (12, 13)

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[2] NSC-45388 was supplied by Drug Research and Development (formerly Cancer Chemotherapy National Service Center), Chemotherapy, National Cancer Institute.

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and animal tumors (1,14), it was suggested that NSC-22420 could be the active form of NSC-45388. The inhibitory effect of NSC-22420 could be reversed by reduced cysteine or glutathione (12,13), and the coupling of NSC-22420 with sulfhydryl or other reaction sites was suggested as the basis for the pharmacologic action of NSC-45388. Both NSC-45388 and NSC-22420 caused inhibition of DNA synthesis in bacterial (12,13) and mammalian (15) cells.

In this study, the effects of NSC-45388 were studied in tumor tissue. The metabolic disposition of NSC-45388 in tumor tissue was studied with the use of methyl- and 2-^{14}C-labeled drug.

MATERIAL AND METHODS

Tumor-Slice Studies

Human tumor tissue was obtained from cancer patients during surgical treatment. Sarcoma 180 (S180) tumors were removed from mice about 1 week after transplantation. After removal of the tumor, it was immediately immersed in Eagle's Minimal Essential Medium (EMEM) (16) containing 10% fetal calf serum and 5 millimols of glutamine, and quickly transported to the laboratory. The tumor was dissected free from contaminating tissues (blood, pus, and necrotic and normal tissues) and uniform sections were sliced with a Stadie-Riggs microtome (17). The incubation mixture consisted of 5 ml of EMEM-10% fetal calf serum and 5 millimols of glutamine. The assays were performed in duplicate. To study the effects of NSC-45388, the drug was added to the media at a final concentration of 5×10^{-4} M unless specified otherwise. To assay for DNA synthesis, 0.5 μmol each of deoxyadenosine, deoxyguanosine, and deoxycytidine, and 5 microcuries of methyl-^{3}H-labeled thymidine (specific activity, 6.7 curie/millimol; purity >98%; New England Nuclear Corp.) were added. For RNA synthesis, 0.5 μmol each of adenosine, guanosine, and cytidine, and 10 microcuries of 5-^{3}H-labeled uridine (specific activity, 4 curie/millimol; purity >98%; Schwartz/Mann) were added. For protein synthesis, 1 microcurie of uniformly labeled ^{14}C-phenylalanine (specific activity, 65 millicurie/millimol; purity >99%; Schwartz/Mann) was added. The tissues were protected from light and incubated for 2 hours at 37 C. They were then chilled, the media decanted, and the tissues homogenized in 5 ml of cold 0.2N perchloric acid. The resultant precipitate was washed twice with 0.2N perchloric acid and twice with ethanol-ether (3:1, v/v).

RNA was hydrolyzed by heating the precipitate in 1.0 ml of 0.3N KOH at 37 C for 1 hour. DNA and protein were precipitated by the addition of 1.0 ml of 0.6N perchloric acid. DNA was extracted from the precipitate by heating in 10% NaCl (pH 7.5) for 30 minutes at 100 C. Protein was extracted by heating the final precipitate in 5 ml of 1N NaOH for 30 minutes at 100 C. Radioactivity was determined by adding 1.0-ml aliquots to 10 ml of Insta-Gel (Packard Instruments Co., Inc., Downers Grove, Ill) and 2 ml of H_2O and counting in a liquid scintillation counter. RNA and DNA were quantitated by absorbancy measurements at 260 nm, and protein was estimated by the Lowry procedure (18). Specific activity of proteins was expressed as counts per minute per milligram, or, in the case of nucleic acids, as counts per minute per unit absorbance at 260 nm.

To test the effect of various concentrations of NSC-45388 on macromolecular synthesis, the drug concentration ranged from 5×10^{-4} to 5×10^{-8} M.

Tissue distribution of methyl- and 2-^{14}C-labeled NSC-45388 was studied in tumor tissue (1 g) which was homogenized in 4 ml of EMEM containing 10% fetal calf serum, 5 millimols of glutamine, 0.4 millimol of nicotinamide adenine dinucleotide phosphate, reduced form (NADPH), and 12.5 microcuries of either methyl- or 2-^{14}C-labeled NSC-45388 (5 millicurie/millimol, purity >99%). The mixture was protected from light and incubated in a shaking water bath at 37 C for 1 hour. Effluent gas was collected for radioactive CO_2, which was assayed according to Woeller (19). Acid-soluble, lipid, RNA, and DNA fractions were prepared as described above and assayed for radioactivity. The amount of ^{14}C in the final protein precipitate was determined by liquid scintillation counting of CO_2 after combustion with oxygen.

The effect of photodecomposition on the interaction of NSC-45388 (or products derived from it) with nucleic acids was studied by add-

ing either methyl- or 2-^{14}C-labeled NSC-45388 (0.012 microcurie) to calf thymus DNA or yeast transfer-RNA (2.5 mg in 5.0 ml of 0.01 M phosphate buffer, pH 7.4), and exposing the mixture to long-wave ultraviolet light (peak, 366 nm). Aliquots were removed at 0, ½, and 1 hour, and the nucleic acids were precipitated with 4 volumes of 95% ethanol-3% potassium acetate. The precipitates were redissolved and reprecipitated three more times. The final precipitates were dissolved in 0.14 M NaCl and aliquots were taken for liquid scintillation counting. The soluble fractions were analyzed by combining the supernatant fluid and washing and evaporating to dryness.

The residues were dissolved in 50% ethanol, and aliquots were spotted on silica gel thin-layer plates (Brinkmann 5762). The plates were developed in 5% $(NH_4)_2SO_4$-5% isopropanol (1:1, v/v) for 3½ hours (20). The R_f values were as follows: 0.32 for NSC-45388, 0.29 for 2-azahypoxanthine, and 0.77 for NSC-7784.

S180 tumors were removed from five or six mice, and microsomes were isolated by homogenizing the tumors in 0.154 M KCl-0.1 M phosphate buffer (pH 7.4) and centrifuging at 10,000 g for 20 minutes. The fatty material was discarded and the supernatant fluid was centrifuged at 105,000 g for 60 minutes. The pellet was suspended in buffer and centrifuged at 105,000 g for 30 minutes. The final pellet was suspended in KCl solution. The incubation mixtures were set up in duplicate. They contained microsomes (equivalent to 1–2 g of tumor), 0.65 μmol of NADP, 6.0 μmol of glucose-6-phosphate, 50 μmol of nicotinamide, 7 units of glucose-6-phosphate dehydrogenase (EC 1.1.1.49), 25 μmol of $MgCl_2$, 1.0 mg of calf thymus DNA, 50 μmol of NSC-45388, and 6.0 microcuries of either methyl- or 2-^{14}C-labeled NSC-45388 in a final volume of 5 ml. The incubation was at 37 C in the absence of light.

For ^{14}C-labeled HCHO analysis, 0.45 μmol of semicarbazide hydrochloride was added to the media. After incubation, protein was precipitated with one-fourth volume of 20% $ZnSO_4$ and saturated $Ba(OH)_2$. Five μmol of carrier HCHO and 0.1 ml of 0.125% 2,4-dinitrophenylhydrazine hydrochloride was added to 2.0 ml of the supernatant fluid. The resultant precipitate was removed by centrifugation and dissolved in benzene. An aliquot was applied to silica gel thin-layer plates and developed in petroleum ether-ethyl ether (40:60, v/v) for 45 minutes. The R_f value for 2,4-dinitrophenylhydrazone of HCHO was 0.36; NSC-45388 and NSC-7784 remained at the origin.

The incubation mixtures were also analyzed for acid-soluble and nucleic acid fractions. The acid-soluble fraction was chromatographed on silica gel thin-layer plates and developed with $(NH_4)_2SO_4$-isopropanol as previously described. The nucleic acids were separated into transfer-RNA, ribosomal RNA, and DNA by chromatography on methylated albumin-kieselguhr (21). After determining the specific activity of each fraction, they were hydrolyzed in 1N HCl and the hydrolysates were chromatographed on paper, using solvent 3 of Markham and Smith (22).

Zonal radioactivity profiles of thin-layer plates were made by scraping 1-cm increments along the length of the chromatogram and placing each fraction in a vial. The absorbent was moistened with 0.5 ml of NCS Solubilizer (Amersham/Searle, Des Plaines, Ill), and 10 ml of the scintillation mixture of Van der Laarse (23) was added and the vials were counted in a liquid scintillation counter.

Methylating properties of NSC-45388 and 5-(3-monomethyl-1-triazeno)imidazole-4-carboxamide (NSC-407347; MIC) were assessed by the method of Epstein et al (24). NSC-407347 was synthesized according to Shealy and Krauth (25). A mixture consisting of 1.0 ml of 0.1 M acetate buffer (pH 4.4), 1.0 ml of 5% 4-(4-nitrobenzyl)pyridine in acetone, and 3 ml of H_2O containing 10 μmol of either NSC-45388 or NSC-407347 was heated to 100 C for 20 minutes. After cooling, 4 ml of acetone and 1 ml of 1 M K_2CO_3 were added, and absorption spectra of the final solutions were obtained in the visible range.

RESULTS

In table 1 it is shown that NSC-45388 at a concentration of 5×10^{-4} M caused a marked reduction in the incorporation of ^{3}H-labeled uridine into RNA in both S180 and human

tumor slices. NSC-45388 inhibited the ^{3}H-labeled thymidine uptake by DNA in S180 tumors but had no observable effect in human tumors. The lack of results in human tumors was probably because they incorporated little ^{3}H-labeled thymidine (about one tenth as much as S180 tumors). Therefore, it was difficult to detect changes in activity with any degree of precision. There was little effect of NSC-45388 on ^{14}C-labeled phenylalanine incorporation into protein in both S180 and human tumors. With S180 tumors, the incorporation by RNA and DNA of their respective ^{3}H-labeled precursors varied proportionately with the negative log of the NSC-45388 concentration in the range studied: 5×10^{-6} to 5×10^{-4} M (fig 1).

The distribution of ^{14}C from either methyl- or 2-^{14}C-labeled NSC-45388 in S180 and human melanoma tumors is summarized in table 2. In all cases, approximately 99.5% of the total activity remained in the acid-soluble fraction, with the remainder being distributed among the lipid, RNA, DNA, and protein fractions (the least amount in the latter). Analyses of acid-soluble fractions by thin-layer chromatography showed that radioactivity was associated mainly with NSC-45388 (when either methyl- or 2-^{14}C-labeled NSC-45388 was used) and NSC-7784 (when 2-^{14}C-labeled NSC-45388 was used). Small amounts of radioactive CO_2 were obtained with methyl-^{14}C-labeled NSC-45388 but not when the 2-^{14}C-labeled compound was used. There was no great difference in the distribution of ^{14}C between S180 and human tumor tissues.

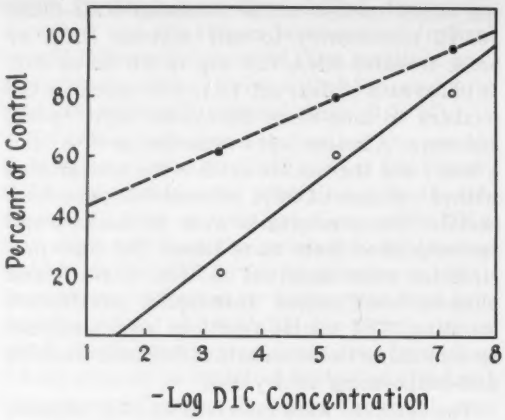

FIGURE 1.—Effect of NSC-45388 (DIC) concentration on incorporation of ^{3}H-labeled thymidine into DNA (○———○) and ^{3}H-labeled uridine into RNA (●.....●) of S180 tumor. Each point represents an average of 2 analyses. (Control specific activity for ^{3}H-labeled thymidine uptake by DNA was 908, and the corresponding value for RNA was 627.)

When solutions containing NSC-45388 and either transfer-RNA or calf thymus DNA were exposed to long-wave ultraviolet light, both methyl- and 2-^{14}C-labeled NSC-45388 disappeared rapidly at similar rates until the level was down to zero after 1 hour (fig 2). There was a concomitant rise in the radioactivity of 2-azahypoxanthine when NSC-45388 was labeled in the 2-position but not when it was labeled in the methyl position. No radioactivity could be detected in RNA or DNA which had been ex-

TABLE 1.—Effect of NSC-45388 on incorporation of radioactive precursors by DNA, RNA, and protein of tumor*

Type of tumor tissue	Source	Percent of control specific activity		
		DNA	RNA	Protein
Lung	Human	85	70	93
Lung	Human	100	47	98
Lung	Human	96	58	—
Rectum	Human	121	52	—
Thymus	Human	90	22	72
S180†	Mice	56 ± 6	63 ± 8	109 ± 4

*Concentration of NSC-45388 used was 5×10^{-4} M. The average control values for human tumors were 28, 297, and 292 for DNA, RNA, and protein respectively. The corresponding control values for S180 tumors were 283, 360, and 415 respectively.

†Values are means of 4 experiments ± SE of mean.

TABLE 2.—Distribution of ^{14}C from labeled NSC-45388 in tumor tissue

Tissue fraction	Percent of total ^{14}C			
	Human melanoma tumor		S180 tumor	
	Methyl-^{14}C	2-^{14}C	Methyl-^{14}C	2-^{14}C
Acid-soluble	99.46	99.50	99.59	99.42
Lipid	0.186	0.127	0.106	0.107
RNA	0.321	0.168	0.189	0.177
DNA	0.025	0.131	0.074	0.253
Protein	0.011	0.067	0.020	0.048
CO_2	0.001	0.000	0.004	0.000

posed to photodecomposition products of either methyl- or 2-^{14}C-labeled NSC-45388.

When microsomal fractions from S180 were incubated with calf thymus DNA and ^{14}C-labeled NSC-45388, there was production of radioactive HCHO only when methyl-^{14}C-labeled compound was used (table 3). On the other hand, radioactive NSC-7784 was formed only when 2-^{14}C-labeled NSC-45388 was present. Approximately equal amounts of radioactivity were recovered in NSC-7784 and HCHO. Table 4 shows the results from nucleic acid fractions which were isolated by column chromatography on methylated albumin kieselguhr. It can be seen that all nucleic acids (transfer-RNA, DNA, and ribosomal RNA) became labeled when they were reacted with methyl-^{14}C-labeled NSC-45388, and there was negligible radioactivity when the 2-^{14}C-labeled compound was used. The labeled nucleic acid fractions were hydrolyzed. The hydrolysis products were resolved by repeated chromatography on paper, and a radioactive uv absorbing compound which co-chromatographed with 7-methylguanine was isolated. This was similar to the results previously reported by Skibba and Bryan for normal rat tissue (11).

NSC-45388 did not react directly with 4-(4-nitrobenzyl)pyridine, a sensitive reagent for the detection of alkylating agents, even under exposure to long-wave ultraviolet light. However, when NSC-407347, an *N*-demethylated product of NSC-45388, was used, a colored pro-

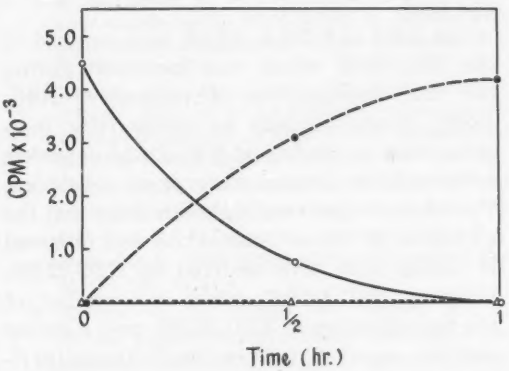

FIGURE 2.—Conversion of NSC-45388 to 2-azahypoxanthine in the presence of light (366 nm) and its effect on binding of NSC-45388 to RNA or DNA. Methyl-^{14}C-labeled NSC-45388 or 2-^{14}C-labeled NSC-45388 (○————○); ^{14}C-labeled 2-azahypoxanthine from 2-^{14}C-labeled NSC-45388 (●.....●); ^{14}C-labeled 2-azahypoxanthine from methyl-^{14}C-labeled NSC-45388 (△ .....△). No radioactivity could be detected in RNA or DNA which had been exposed to photodecomposition products of either methyl- or 2-^{14}C-labeled NSC-45388. Each point represents an average of 2 results.

TABLE 3.—Metabolism of ^{14}C-labeled NSC-45388 by S180 microsomal fraction

Labeled NSC-45388	Total counts per minute	
	HCHO	NSC-7784
Methyl-^{14}C	1256	0
2-^{14}C	0	1126

TABLE 4.—Alkylation of nucleic acids by NSC-45388*

Tissue fraction	Counts per minute per mg of nucleic acid	
	Methyl-^{14}C	2-^{14}C
Transfer-RNA	120	0
DNA	340	2
Ribosomal RNA	520	0

*Microsomes from S180 tumors were incubated with radioactive NSC-45388 and calf thymus DNA for 4 hours in the absence of light.

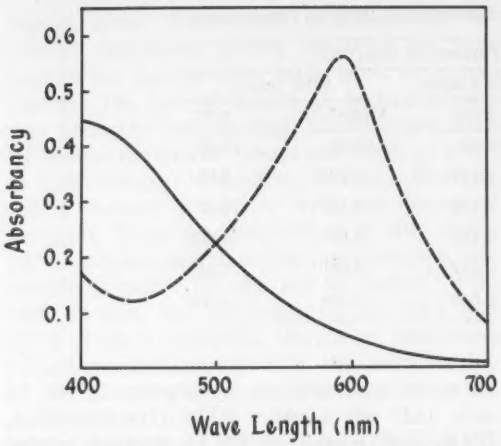

FIGURE 3.—Absorption spectra of products of reaction of 4-(4-nitrobenzyl)pyridine with NSC-45388 (————) and with NSC-407347 (.....).

duct was formed which was characteristic of the salt of the alkylated 4-(4-nitrobenzyl)-pyridine (fig 3). NSC-407347 was reported to be highly unstable in aqueous solution (25). NSC-7784 was identified as one of its products of decomposition. The other product is presumed to be diazomethane or a carbonium ion which possesses the properties of an alkylating agent.

DISCUSSION

In S180 tumor slices, NSC-45388 inhibited the uptake of radioactive precursors by RNA and DNA, and the inhibition was proportional to the concentration of NSC-45388. NSC-45388 also inhibited the incorporation of RNA precursors in human tumor tissue. Protein synthesis was less sensitive to the effects of NSC-45388, since it was not inhibited by levels of NSC-45388 which affected nucleic acids. The distribution of ^{14}C from methyl- and 2-labeled NSC-45388 in various tumor fractions (acid-soluble, lipid, nucleic acid, and protein) was similar in S180 and human melanoma tumors. With tumor microsomal preparations, it was shown that ^{14}C-labeled HCHO was derived only from methyl-labeled NSC-45388, and ^{14}C-labeled NSC-7784 was found only when 2-labeled NSC-45388 was used. The nucleic acids in the incubation mixture became labeled only with the methyl-labeled drug, and ^{14}C-labeled 7-methylguanine was isolated from the nucleic acid hydrolysates. These results showed that NSC-45388 was metabolized by tumor tissue in a manner similar to that of normal tissue and according to the scheme proposed by Preussman et al (26) and Skibba et al (8). It was proposed that NSC-45388 is first demethylated to form an intermediate, presumably NSC-407347, which decomposes to form NSC-7784 and a potential methylating agent.

It has been postulated that NSC-22420 may be the pharmacologically active form of NSC-45388 (12,14). The ease with which diazo compounds can couple with a number of reactive groups was suggested as the basis for their mechanism of action. NSC-45388 is unstable in ultraviolet light of long-wave length (27). An end product of the reaction is 2-azahypoxanthine, which is presumed to arise from the spontaneous cyclization of NSC-22420, which is a photodecomposition product of NSC-45388. When radioactive NSC-45388 was incubated with DNA in the presence of long-wave ultraviolet light, the 2-azahypoxanthine was labeled only when 2-^{14}C-labeled NSC-45388 was used.

The RNA and DNA, which were exposed to the NSC-22420 which was generated during the photodecomposition of radioactive NSC-45388, showed virtually no radioactivity, indicating that no binding of NSC-22420 to nucleic acids could be detected under these conditions. Therefore, it appeared highly unlikely that the alkylation of nucleic acids which was observed in tissues could have occurred via NSC-22420. However, NSC-407347, which is a product of the demethylation of NSC-45388, gave a strong positive reaction with 4-(4-nitrobenzyl)pyridine, a reagent which is useful for the detection of alkylating agents. Even in the presence of light, NSC-45388 did not produce a positive test with this reagent. Therefore, the evidence indicated that in tumor tissue in vitro, the alkylation of nucleic acids did not occur via NSC-22420 but involved the metabolic pathway in which NSC-407347 is formed as an intermediate.

Whether methylation of DNA can adequately

account for the inhibitory effect of NSC-45388 on the synthesis of RNA and DNA in vitro remains to be clarified. Mammalian cells have been shown to be capable of removing alkyl groups from DNA and can carry out repair synthesis of DNA (28). However, since methylated DNA was shown to lose its ability to participate in bacterial transformation (29) and had reduced template activity for RNA synthesis (30), there could be enough damage induced by methylation to interfere with the biologic function of DNA. Methylation of DNA has been reported to produce single-strand breaks either directly or by enzyme action (29), and it is conceivable that lesions may be of sufficient magnitude to diminish the ability of the altered DNA to act as template for RNA or DNA synthesis, or both.

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Effect of Different Doses of Methotrexate (NSC-740), Cytosine Arabinoside (NSC-63878), and Cyclophosphamide (NSC-26271) on Drug Resistance in Mice with L1210 Leukemia [1,2,3]

Franz A. Schmid and Dorris J. Hutchison [4]

SUMMARY

The influence of dosage on the development of resistance to methotrexate, cytosine arabinoside, and cyclophosphamide was studied in the L1210 (V) mouse leukemia system. Resistance to methotrexate developed more rapidly in the L1210 sublines in mice receiving the drug at the higher dose levels, but it was not in direct proportion to the dose. In general, resistance to cytosine arabinoside developed faster after six daily injections of 10 mg/kg of the drug than after six daily injections of 80 mg/kg. Cyclophosphamide was administered in a single dose of 200 mg/kg or in multiple smaller doses. Resistance, which occurred earlier as the result of the latter dosage, was incomplete after both schedules. Resistance to methotrexate and cytosine arabinoside, however, was complete (100%). High doses of all three drugs produced some sublines which had increased survival times compared with the sensitive parent line L1210 (V).

[Cancer Chemother Rep 56:473–481, 1972]

Since modification of dosage greatly influences the effect of a drug on tumor growth, and since drug resistance is a major obstacle to successful treatment, various dose schedules should be investigated and their influence on the production of resistance determined.

The experiments reported herein were designed to study the effect of changes in drug dosage on resistance in mice with L1210 leukemia. Two antimetabolites, methotrexate and cytosine arabinoside, and one alkylating agent, cyclophosphamide, were studied. These drugs are important in clinical use and have been widely investigated in the L1210 system. Methotrexate and cytosine arabinoside are cell cycle phase-specific agents (1–3). Cytosine arabinoside inhibits cell proliferation in the S phase specifically while methotrexate inhibits RNA and protein synthesis as well as DNA synthesis. Their antitumor action is increased when a high external concentration is maintained long enough for a large number of tumor cells to be exposed (1,2,4). Cyclophosphamide, a cell cycle phase-nonspecific agent, is highly cytotoxic to both dividing and nondividing cells (5–7).

MATERIALS AND METHODS

L1210 (V) mouse leukemia and its resistant

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[2] Supported in part by grant CA-08748 from the National Cancer Institute, National Institutes of Health, and grant T-107 from the American Cancer Society.

[3] Methotrexate: glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-; MTX.

Cytosine arabinoside: cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride; ara-C.

Cyclophosphamide: 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate; Cytoxan; CTX.

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sublines were maintained in BDF_1 (C57BL/6 female × DBA_2 male) mice obtained from A. R. Schmidt Co., Madison, Wisconsin. The treated and control groups consisted of five or ten mice weighing 19–23 g.

The chemotherapeutic agents were prepared as follows: cytosine arabinoside, cyclophosphamide, and NSC-135758 (8) were dissolved in saline; methotrexate was dissolved in saline and sodium bicarbonate; and carbazilquinone (9) was dissolved in dimethylsulfoxide and then diluted with saline.[5]

L1210 leukemic cells (10^6) were implanted intraperitoneally (ip) and treatment was started 1 day later. In general, the chemotherapeutic compounds were administered ip in a volume of 0.01 ml/g of mouse body weight.

For the production of the three sublines resistant to methotrexate, mice received either 0.75 mg/kg (subline A), 3.0 mg/kg (subline B), or 12.0 mg/kg (subline C) of the drug every other day for three injections. All transfer generations of each subline were treated.

Ten sublines resistant to cytosine arabinoside were produced. Of the ten groups of mice in which the sublines were produced, five groups received 10 mg/kg and five received 80 mg/kg of the drug in six daily doses.

The development of resistance to cyclophosphamide was studied in eight sublines. All transfer generations of every line were treated with a total of 200 mg/kg of mouse body weight except one which was treated with a total of 400 mg/kg. Thus, dose schedules varied from a single dose of 200 mg/kg to eight daily doses at 25 mg/kg. (The single dose was always included for reference.)

Specific treatments were given in single injections or in divided doses on alternate days until ten injections had been given or until 50% of the mice in a group had died. Results are based on the median survival time (MST) of the individual groups of mice.

RESULTS

Resistance to Methotrexate

Methotrexate was tested over a wide range of dosages against L1210 (V) and ten transfer generations and, subsequently, the tenth untreated transfer generation (*see* table 1). The results indicated a relationship between drug dose and response. Such titration data provided a means of quantitating the methotrexate-resistance levels over a twelvefold range. Against the parental line, the effects of single doses of cyclophosphamide, carbazilquinone, and NSC-135758 were similar.

[5] NSC-135758: 2,5-piperazinedione, 3,6-bis-(5-chloro-2-piperidyl)-, dihydrochloride; compound 593-A. A product of Streptomyces fermentation with alkylating activity. Gitterman, C. Personal communication.

Carbazilquinone: NSC-134679; *p*-benzoquinone, 2,5-bis(1-aziridinyl)-3-(2-hydroxy-1-methoxyethyl)-6-methyl-, carbamate (ester).

TABLE 1.—Effect of three different doses of methotrexate on the development of resistance in mice with L1210 leukemia

Transfer generation	Drug	Dosage (mg/kg)*	Median survival time (days) of mice carrying the subline— A	B	C
Methotrexate-treated					
1	Control†	—	8	8	8
	Methotrexate	0.75‡	10	—	—
		3‡	—	13	—
		12‡	—	—	16
		0.75	11	—	—
		3	17	—	—
		6	21	—	—
		9	22	—	—
	Cyclophosphamide	200 × 1	17	—	—

TABLE 1.—Effect of three different doses of methotrexate on the development of resistance in mice with L1210 leukemia—Continued

Transfer generation	Drug	Dosage (mg/kg)*	Median survival time (days) of mice carrying the subline— A	B	C
Methotrexate-treated					
	Carbazil-quinone	2 × 1	17	—	—
	NSC-135758	10 × 1	17	—	—
2	Control	—	8	8	8
	Methotrexate	0.75‡	10	—	—
		3‡	—	11	—
		12‡	—	—	13
		0.75	11	10	9
		3	13	13	10
		6	19	16	13
		9	20	18	14
2	Control	—	8	8	8
	Methotrexate	0.75‡	10	—	—
		3‡	—	10	—
		12‡	—	—	10
		0.75	10	9	9
		3	12	11	10
		6	14	12	11
		9	15	13	11
	Cyclo-phosphamide	200 × 1	17	18	17
	Carbazil-quinone	2 × 1	15	16	15
	NSC-135758	10 × 1	16	18	16
4	Control	—	8	8	9
	Methotrexate	0.75‡	8	—	—
		3‡	—	9	—
		12‡	—	—	10
		0.75	9	9	9
		3	12	10	10
		6	13	12	10
		9	13	12	10
	Cyclo-phosphamide	200 × 1	16	17	17
	Carbazil-quinone	2 × 1	16	16	> 30
	NSC-135758	10 × 1	18	16	25
5	Control	—	8	8	14
	Methotrexate	0.75‡	9	—	—
		3‡	—	9	—
		12‡	—	—	10

TABLE 1.—Effect of three different doses of methotrexate on the development of resistance in mice with L1210 leukemia—Continued

Transfer generation	Drug	Dosage (mg/kg)*	Median survival time (days) of mice carrying the subline— A	B	C
Methotrexate-treated					
	Methotrexate	0.75	9	9	10
		3	11	10	10
		6	12	11	10
		9	12	11	10
	Cyclophosphamide	200 × 1	17	16	16
	Carbazilquinone	2 × 1	16	16	30
	NSC-135758	10 × 1	18	17	29
7	Control	—	8	9	11
	Methotrexate	0.75‡	8	—	—
		3‡	—	9	—
		12‡	—	—	9
		0.75	9	9	9
		3	10	9	9
		6	10	10	9
		9	10	10	9
8	Control	—	8	9	10
	Methotrexate	0.75‡	8	—	—
		3‡	—	9	—
		12‡	—	—	10
		0.75	9	9	10
		3	9	9	10
		6	9	9	10
		9	9	9	10
11	Control	—	8	9	10
	Methotrexate	0.75‡	8	—	—
		3‡	—	9	—
		12‡	—	—	10
		0.75	8	9	10
		3	8	9	10
		6	8	9	10
		9	8	9	9
	Cyclophosphamide	200 × 1	15	14	19
	Carbazilquinone	2 × 1	19	19	22
	NSC-135758	10 × 1	17	20	24

TABLE 1.—Effect of three different doses of methotrexate on the development of resistance in mice with L1210 leukemia—Continued

Transfer generation	Drug	Dosage (mg/kg)*	Median survival time (days) of mice carrying the subline— A	B	C
Untreated					
10	Control	—	8	8	8
	Methotrexate	0.75	8	8	8
		3	8	9	9
		6	10	9	9
		9	10	10	10

*Therapy which was started 1 day after ip inoculation with 10^6 cells was given either as a single injection or in divided doses on alternate days until ten injections had been given or 50% of the mice in a group had died.

†Sensitive parent line L1210 (V).

‡Dose used to develop resistance. For each treated transfer generation, mice carrying subline A received 0.75 mg/kg, mice carrying subline B received 3.0 mg/kg, and mice carrying subline C received 12.0 mg/kg every other day for three injections.

Resistance to methotrexate increased more rapidly in the two sublines in mice receiving the higher doses. In the third generation of sublines A, B, and C in mice receiving the respective doses of 0.75, 3.0, and 12.0 mg/kg, resistance increased about threefold, fivefold, and eightfold, respectively, compared with the sensitive line (L1210[V]). These increases were obtained by comparing the effects of the 0.75-, 3-, 6-, and 9-mg/kg doses on a treated transfer generation with the effects on the parent line (generation 1, table 1). There was little, if any, change in sensitivity to the three alkylating agents (cyclophosphamide, carbazilquinone, NSC-135758).

For the subline in mice receiving 12 mg/kg of methotrexate in the fourth transfer generation, the MST of the untreated controls was 9 days and the resistance to methotrexate was almost complete. In the fifth generation, the MST of the untreated controls increased to 14 days, but in the eighth generation, it dropped to 10 days. The change in the MST of the controls was accompanied by a noticeable increase in sensitivity, ie, collateral sensitivity to carbazilquinone and NSC-135758. Mice carrying the resistant lines had increased survival times after treatment with these two drugs.

In the seventh transfer generation, resistance was almost complete in the subline in mice receiving 3 mg/kg of methotrexate; the MST of the untreated controls was 9 days.

After the three methotrexate-resistant sublines had been maintained in untreated mice for ten generations they again became slightly sensitive to the higher doses of methotrexate.

Resistance to Cytosine Arabinoside

The effect of different doses of cytosine arabinoside on the selection of resistance in L1210 (V) variants is shown in table 2. Five groups of mice (carrying five sublines) received 10 mg/kg of cytosine arabinoside and five groups (carrying five other sublines) received 80 mg/kg in six daily doses. Of the sublines in mice receiving doses of 10 mg/kg, one subline was completely resistant in the second transfer generation, two were resistant in the third generation, and two were resistant in the fourth generation. In sublines carried by mice receiving 80 mg/kg, however, resistance developed more slowly. Two sublines were completely resistant in the third transfer generation, one was resistant in the fourth generation, one was resistant in the eighth generation, and one was resistant in the ninth generation.

TABLE 2.—Effect of two different doses of cytosine arabinoside on the development of resistance in mice with L1210 leukemia

Cytosine arabinoside-treated transfer generation	Cytosine arabinoside* (mg/kg)	Median survival time (days) of cytosine arabinoside-treated mice carrying subline—									
		A†	B†	C†	D†	E†	F‡	G‡	H‡	I‡	J‡
1	0	8	8	8	8	8	8	8	8	8	8
	10	11	15	14	14	16	15	15	12	14	15
	40	15	16	17	13	16					
	80	15	15	14	14	17	15	14	15	13	16
2	0	7	8	8	8	8	7	8	8	8	8
	10	7	11	16	15	16	15	10	15	14	16
	40	8				14	14				16
	80	8	11	11	14	14	14	9	15	14	16
3	0		8	8	8	8	7	8	8	7	8
	10		8	8	10	10	7	8	11	13	15
	40					9	7				15
	80		8	8	9	8	7	8	11	14	15
4	0				8	8			8	8	8
	10				8	8			8	15	14
	40					8					14
	80				8	8			8	15	13
8	0									10	8
	10									9	14
	40										14
	80									7	13
9	0										8
	10										8
	40										8
	80										8

*Treatment, which was started 1 day after ip inoculation with 10^6 leukemic cells, was given in six daily injections.

†Treatment: 10 mg/kg, six daily injections.

‡Treatment: 80 mg/kg, six daily injections.

Further studies revealed that the untreated control mice for the subline resistant at the eighth generation had an MST of 10 days and that the groups treated with injections of 3 or 6 mg/kg of methotrexate on alternate days for ten injections or with a single dose of 200 mg/kg of cyclophosphamide had MSTs of more than 30 days.

Resistance to Cyclophosphamide

An MST of 17 days was noted after the administration of a single dose of 200 mg/kg of cyclophosphamide to mice bearing the sensitive L1210 (V) line in the first transfer generation (table 3). The MST after the administration of 25 mg/kg × 8 was 12 days and selection of resistance was more rapid. In the sixth generation, the MSTs for mice treated with the three higher doses were 14, 14, and 19 days; for mice treated with the lower doses, the MSTs were only 10 or 11 days; and for mice treated with the respective reference doses of 200 mg/kg, the MST was 12 days. By the 11th treatment generation, the MSTs of the five groups of mice that received the lower doses were about the same as those of the mice carrying sixth generation cells; however, the three sublines in the mice given the single doses of 200 mg/kg behaved less uniformly. None of the eight cyclophosphamide-"resistant" lines were completely resistant. Although the MST of the cyclophosphamide-treated group carrying L1210/cyclophosphamide subline B was less than that of the controls, the subline could not be considered totally resistant because the MST of the untreated controls had increased to 12 days.

TABLE 3.—Effect of different doses and treatment schedules of cyclophosphamide on the development of resistance in mice carrying L1210 leukemia

Subline	Cyclophosphamide (mg/kg, daily)	Number of treated transfer generations 1	2	6	11	21
		Median survival time (days)				
	Untreated controls	8	9	9	9	10
A	200 × 1	17	22	14	11	12
	Untreated controls	8	9	10	12	12
B	200 × 1	17	15	14	10	10
	Untreated controls	—	9	10	9	9
C	200 × 1	—	20	19	14	13
	Untreated controls	8	8	8	8	
D	100 × 2	16	14	10	9	
	200 × 1	—	15	12	10	
	Untreated controls	8	8	8	9	
E	50 × 8	13	13	11	9	
	200 × 1	—	15	12	10	
	Untreated controls	8	8	8	8	
F	50 × 4	12	11	10	10	
	200 × 1	—	16	12	12	
	Untreated controls	8	8	9	9	
G	25 × 8	12	10	10	10	
	200 × 1	—	18	12	12	
	Untreated controls	8	8	8	8	
H	25 × 8	12	10	10	9	
	200 × 1	—	14	12	12	

In an additional study on cross resistance, it was found that after 11 treatment passages with cyclophosphamide, the eight lines of L1210 (V) were fully sensitive to methotrexate and moderately cross resistant to carbazilquinone and NSC-135758.

DISCUSSION

With increasing doses, resistance to methotrexate developed earlier but was not in direct proportion to the dose. The subline in mice receiving 12 mg/kg became resistant almost twice as rapidly as the variant in mice receiving 0.75 mg. All three sublines regained only a slight fraction of their original sensitivity to methotrexate after passage in untreated mice. This finding is in agreement with the results of Hoshino et al (10).

Unexpectedly, resistance to cytosine arabinoside generally developed more rapidly after six daily injections of 10 mg/kg than after six daily injections of 80 mg/kg. The literature revealed similar examples. Dixon and Adamson (11) could produce resistance to cytosine arabinoside at will in one generation of L1210 with daily subcutaneous (sc) injections of 20 mg/kg of the drug. Wodinsky and Kensler (12), however, reported that their L1210 line was not completely resistant to cytosine arabinoside (5 daily doses of 160 mg/kg) until after ten transfer generations (total dose, 8000 mg/kg). Schabel[6] produced L1210 variants solidly resistant to cytosine arabinoside in one generation with multiple treatment courses of only 12.5 mg/kg (total dose, 400 mg/kg). It is paradoxical that the lower rather than the higher doses of cytosine arabinoside caused the more rapid selection of resistance despite the fact that an increase in the dose of an antimetabolite is not followed by a directly proportionate increase in cell kill (2). The reasons for this discrepancy in resistance development with low and high doses of cytosine arabinoside are being further explored.

None of the eight cyclophosphamide-"resistant" sublines were completely resistant. The MST of mice carrying the resistant subline of Lane and Yancey (13,14) treated with single doses of cyclophosphamide (200 mg/kg) did not decrease to the MST of the controls during 20 treated transfer generations; the subline retained about 20% of its original sensitivity to cyclophosphamide. Schmidt (15,16) suggested a common type of antitumor activity among the diverse alkylating agents, irrespective of their structural variability. His melphalan-resistant Dunning leukemia line was completely resistant to melphalan and was cross resistant to the various alkylating agents used.[7] Our L1210 leukemia line, however, did not develop complete resistance to cyclophosphamide and cross resistance to other alkylating agents was only moderate at best. In contrast to resistance to cyclophosphamide, resistance to methotrexate and cytosine arabinoside was complete and cross resistance was not evident.

It is of interest that the high doses of all three drugs resulted in sublines with increased MSTs compared with the parent line L1210 (V). Similar instances have been reported (17, 18). Humphreys and Goldin (19) recovered tumors with decreased growth rate from mice bearing L1210 treated with antifolates. Johnson et al (20) reported that after treatment with cyclophosphamide several sublines were substantially modified with respect to virulence and karyotype (compared with the parent line). This phenomenon is the subject of further investigation.

[6] Schabel, F. M., Jr. Personal communication.

[7] Melphalan: NSC-8806; alanine, 3-[*p*-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, L-; L-phenylalanine mustard.

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Studies of the L1210 Antileukemic Activity of *O*-2,2′-cyclocytidine, Monoacetate (Anhydro-ara-C; NSC-129220)—Comparison With Cytosine Arabinoside (NSC-63878) With Respect to Treatment Schedule Dependency [1,2,3,4]

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SUMMARY

O-2,2′-cyclocytidine, monoacetate (anhydro-ara-C) was compared with cytosine arabinoside in a study of the influence of treatment schedule on activity against mouse L1210 leukemia. Typically, cytosine arabinoside produced a substantial number of long-term survivors (>60 days) when given intraperitoneally (ip) every 3 hours on every fourth day (q3h/24h/q4d) to mice with early ip implanted tumor. Alternative schedules of cytosine arabinoside treatment, including treatment once each day, failed to elicit long-term survivors. In contrast, ip treatment with anhydro-ara-C, while as effective as cytosine arabinoside on the q3h/24h/q4d schedule, did produce a substantial number of long-term survivors when given once each day. The advantage of daily anhydro-ara-C treatment over daily cytosine arabinoside treatment was retained when the drugs were given orally. However, neither drug given orally produced a significant number of long-term survivors on any schedule. Daily treatment with anhydro-ara-C was more effective than daily treatment with cytosine arabinoside against advanced L1210 leukemia implanted subcutaneously and against moderately advanced L1210 leukemia implanted intracranially. A variant of L1210 developed for resistance to cytosine arabinoside was cross resistant to anhydro-ara-C. Studies by others suggest that deamination of anhydro-ara-C by mouse kidney pyrimidine nucleoside deaminase is minimal and may account for its apparently longer duration of action compared with cytosine arabinoside.

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[3] Experiments were conducted in the laboratories of the Drug Evaluation Branch (DEB), Drug Research and Development (DR&D), Chemotherapy, NCI; and Microbiological Associates, Inc.

[4] Anhydro-ara-C: 6*H*-furo[2′,3′:4,5]oxazolo[3,2-*a*]pyrimidine-2-methanol, 2,3,3*a*,9*a*-tetrahydro-3-hydroxy-6-imino-, monoacetate (salt). Submitted to DR&D for screening by Dr. Robert Sanches, Salk Institute of Biological Studies, San Diego, Calif.

Cytosine arabinoside: cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride; ara-C. Supplied by DR&D, Chemotherapy, NCI.

[5] DEB, DR&D, Chemotherapy, NCI, Bethesda, Md.

[6] Microbiological Associates, Inc., Bethesda, Md.

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The effectiveness of cytosine arabinoside (fig 1) against L1210 leukemia in mice has been shown to be profoundly influenced by the treatment schedule and the extent of disease when treatment is begun (1–4). Against early L1210, cytosine arabinoside treatments given every 3 hours for a 24-hour period every fourth day after tumor implantation (q3h/24h/q4d) have been considerably more effective than alternative schedules including one injection q4d and one injection daily (1–4). The schedule dependency of cytosine arabinoside, which inhibits DNA polymerase as the nucleotide (5), has been attributed to the short doubling time and high proliferative fraction of L1210 (1,2,6). In addition, the duration of action of each cytosine arabinoside treatment has been related to its rate of phosphorylation to the active inhibitor and to its relatively rapid deamination to uracil arabinoside,[8] which is ineffective against L1210 (7–12). The need to administer cytosine arabinoside almost continually on each day of intermittent therapy (eg, q3h/24h/q4d) to attain a substantial number of long-term survivors has led to attempts to develop structural congeners which have similar mechanisms of antitumor action but which might achieve more prolonged effective drug levels in vivo (10,13–15). Three such derivatives, the 5′-palmitate (NSC-135962) (13), the 5′-adamantoate (NSC-117614) (14), and the C-4 modifications of cytosine arabinoside (15)[9] have been found to be very effective against early L1210 when given in one single treatment on the first or second day after tumor implantation, a schedule which is only minimally effective for cytosine arabinoside. However, such agents are insoluble in water or physiologic saline and require preparation as suspensions for injection (13–15). In contrast, cytosine arabinoside hydrochloride is soluble in water at a concentration of 400 mg/ml and

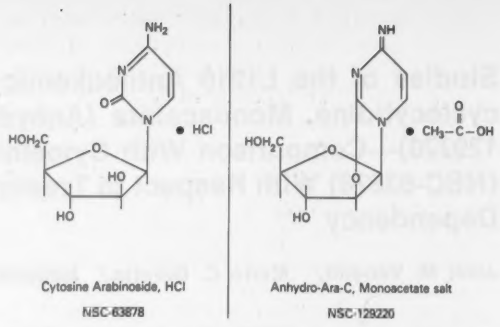

FIGURE 1.—Chemical structures of cytosine arabinoside hydrochloride and anhydro-ara-C monoacetate salt.

solutions are stable for approximately 7 days at refrigerator temperatures (16).

Recently, Hoshi et al (10) reported that *O*-2,2′-cyclocytidine, hydrochloride (NSC-145668)[10] (17), a water-soluble congener of cytosine arabinoside, was considerably more effective than cytosine arabinoside when the drugs were given daily to mice with early L1210: daily treatment with NSC-145668 produced a substantial number of long-term survivors while daily treatment with cytosine arabinoside did not (10). Concurrently, anhydro-ara-C (fig 1) which we have found to be water soluble at concentrations as high as 200 mg/ml, was tested against L1210 in the DR&D screening program and was found to produce a number of long-term survivors when given daily. These observations prompted the present studies of the comparative treatment schedule dependency of anhydro-ara-C in the L1210 test system and of the activity of anhydro-ara-C against a cytosine arabinoside-resistant subline of L1210.

MATERIALS AND METHODS

The methods used were essentially those described previously for screening and evaluation of treatment schedule dependency of various drugs against L1210 leukemia in mice (3,18,19). BDF_1 male mice weighing 18–26 g were used for studies of drug effects against early intraperitoneally (ip) or intracranially (ic) implanted L1210. BDF_1 female mice weighing 18–24 g were used in one experiment involving treatment of mice with subcutaneously (sc)

[8] Uracil arabinoside: NSC-68928; uracil, 1-β-D-arabinofuranosyl-.

[9] NSC-135962: cytosine, 1-β-D-arabinofuranosyl-, 5′-palmitate; palmO-ara-C.

NSC-117614: cytosine, 1-β-D-arabinofuranosyl-, 5′-(1-adamantanecarboxylate).

[10] NSC-145668: 6*H*-furo[2′,3′:4,5]oxazolo[3,2-*a*]pyrimidine-2-methanol, 2,3,3*a*,9*a*-tetrahydro-3-hydroxy-6-imino, monohydrochloride.

implanted L1210. CDF_1 male mice weighing 18–26 g were used in a comparison of drug effects against the parent strain of L1210 and the cytosine arabinoside-resistant variant, L1210/ara-C/R; both tumors were implanted ip. Mice with ip implanted tumors were inoculated on Day 0 with 0.1 ml of 0.85% saline containing 10^5 L1210 or L1210/ara-C/R cells harvested from the ascites of stock tumor-bearing DBA/2 mice. Mice inoculated ic were each given 3×10^5 L1210 cells. For sc implanted L1210, 10^6 cells were implanted into the right hind leg. The drug treatment routes and schedules for each experiment are noted in the tables of results.

Anhydro-ara-C and cytosine arabinoside were dissolved in distilled water. For both, dosage levels up to 2000 mg/kg were effected by injecting solutions of 200 mg/ml in the constant volume of 0.01 ml/g mouse weight. Higher doses were attained by appropriately increasing the volume of 200 mg/ml solution injected. At the 200 mg/ml concentration, anhydro-ara-C (as the monoacetate salt) and cytosine arabinoside (as the hydrochloride) exhibit pH levels of 6.4 and 4.5 respectively. BCNU [11] was used as a positive control in the experiment involving treatment of ic implanted L1210 and was dissolved in 0.85% saline.

RESULTS

The experiment summarized in table 1 shows that when given ip to mice with early ip implanted L1210, optimal daily anhydro-ara-C treatment was superior to optimal daily cytosine arabinoside treatment. Daily treatment with anhydro-ara-C produced three of eight long-term survivors, whereas daily treatment with cytosine arabinoside typically produced none (table 1). On the q3h/24h/q4d schedule, anhydro-ara-C produced four of eight survivors and cytosine arabinoside produced only one of eight survivors, the latter being less effective than usual (1) on this schedule in this experiment. With alternative ip schedules (once, Day 1; q3h, Day 1 only; once, Days 1, 5, and 9), the results of cytosine arabinoside treatment were consistent with previous findings (1) and anhydro-ara-C displayed the same level of activity as cytosine arabinoside. When given daily sc or orally, neither drug produced survivors but anhydro-ara-C was superior to cytosine arabinoside in increasing the median survival time of the mice. The relative ip optimal doses (OD) of the drugs on the various schedules used in the experiment summarized in table 1 were of interest. For example, when the agents were given once on Day 1 only or once q4d, the cytosine arabinoside OD was four times as large as the equally effective OD for anhydro-ara-C. In contrast, when the drugs were given at shorter intervals (q3h, Day 1 only; q3h/24h/q4d and daily, Days 1–9), the OD of anhydro-ara-C was considerably higher than the OD of cytosine arabinoside (table 1). The fivefold greater OD for anhydro-ara-C relative to cytosine arabinoside with daily treatment is consistent with the difference in the daily ODs of the hydrochloride forms of anhydro-ara-C and cytosine arabinoside reported by Hoshi et al (10). In the experiment summarized in table 1, the OD of anhydro-ara-C given on a number of ip schedules, including daily and q3h/24h/q4d, was the highest dose used. The importance of the observation that anhydro-ara-C displayed about the same effectiveness whether given daily or q3h/24h/q4d, prompted more detailed study of these critical schedules. In the experiments summarized in table 2, the 80% increase in survival resulting from cytosine arabinoside treatment q3h/24h/q4d was typical (1), and the superiority of anhydro-ara-C over cytosine arabinoside on the daily treatment schedule was confirmed. Thus, from the data summarized in tables 1 and 2 it is concluded that when given ip to mice with early L1210 implanted ip, (*a*) daily treatment with anhydro-ara-C is superior to daily treatment with cytosine arabinoside, (*b*) q3h/24h/q4d treatment with anhydro-ara-C is as effective as q3h/24h/q4d treatment with cytosine arabinoside, and (*c*) there is little or no difference between the drugs on alternative schedules. More detailed studies of oral drug administration (table 3) showed that both compounds were generally less effective when given orally than when given ip. When given orally, anhydro-ara-C appeared to be somewhat inferior to cytosine arabinoside

[11] BCNU: NSC-409962; urea, 1,3-bis(2-chloroethyl)-1-nitroso-.

TABLE 1.—Influence of treatment schedule on L1210 antileukemic activity of anhydro-ara-C and cytosine arabinoside*

Treatment route and schedule	Anhydro-ara-C				Cytosine arabinoside			
	Dose range (mg/kg/injection)	OD†	ILS‡ (%)	Day 60 S/T§	Dose range (mg/kg/injection)	OD†	ILS‡ (%)	Day 60 S/T§
Ip; once, Day 1	200–1600	800	33	0/8	400–3200	3200	33	0/8
Ip; q3h/24h, Day 1 only	12.5–200	200	55	0/8	5–40	40	55	0/8
Ip; once each day, Days 1,5,9	100–800	800	117	0/8	400–3200	3200	117	0/8
Ip; q3h/24h, Days 1,5,9	12.5–100	100	>378	4/8	5.0–40	20	172	1/8
Ip; daily, Days 1–9	50–400	400	272	3/8	10–80	80	78	0/8
Sc; daily, Days 1–9	50–800	400	167	0/8	10–80	40	106	0/8
Oral; daily, Days 1–9	50–800	400	122	0/8	20–160	160	67	0/8

*Tumor inoculum = 10^5 cells/mouse. Untreated control median survival time (MST) = 9.0 days (0/16 survivors).

†In this and subsequent experiments cytosine arabinoside and anhydro-ara-C were given over a wide range of doses. Comparative antitumor activity was based on the maximum increase in lifespan (ILS) achieved. The dose at which the maximum ILS occurred is defined as the optimal dose (OD). In some instances, the OD was the highest dose used. Previous experience with cytosine arabinoside in this laboratory indicated that there would be no advantage in treating at still higher dosage levels with the particular treatment schedules involved. Where the OD for anhydro-ara-C was observed to be optimal, it is possible that still higher doses might have been even more effective.

‡Percent increase in MST over controls at OD.

§No. of 60-day survivors/total treated.

on the q3h/24h/q4d schedule. However, anhydro-ara-C given orally was clearly superior to cytosine arabinoside given orally when the drugs were given daily (table 3).

In the experiment summarized in table 4, L1210 was implanted sc and sc treatment was delayed until Day 5. Against this greater tumor challenge, cytosine arabinoside itself produced only one of eight survivors on the q3h/24h/q4d schedule. The results for cytosine arabinoside were consistent with previous findings that the drug is generally not curative against advanced L1210 and that one treatment every second or fourth day is as effective as q3h/24h/q4d treatment at their respective ODs, although the OD for single-dose q4d treatment is 12–20 times higher than the total daily OD when the drug is given q3h/24h/q4d (4). Against the advanced tumor, parenterally administered anhydro-ara-C was superior to parenterally administered cytosine arabinoside given daily, but the difference was not as great as had been observed against early L1210 (tables 1, 2, and 4). Against moderately advanced ic implanted L1210, daily ip treatment with anhydro-ara-C was superior to daily ip treatment with cytosine arabinoside and both were inferior to a single ip treatment with BCNU (table 5).

In an attempt to determine the effect of aging in solution on the activity of anhydro-ara-C against L1210, mice with early ip implanted tumor were given daily injections of solutions prepared each day just before injection and their survival times were compared with those of mice receiving daily injections with solutions prepared 24 hours before the first daily treatment (table 6). The latter solutions were refrigerated in brown glass bottles during the 9 days of treatment. The results indicated that the aged solutions retained antileu-

TABLE 2.—Comparative schedule dependency of ip treatment with anhydro-ara-C or cytosine arabinoside against early L1210 leukemia*

Expt No. (control MST in days)	Ip treatment schedule	Dose (mg/kg/injection)	Anhydro-ara-C MST (days)	Anhydro-ara-C ILS† (%)	Anhydro-ara-C Day 60 S/T‡	Cytosine arabinoside MST (days)	Cytosine arabinoside ILS† (%)	Cytosine arabinoside Day 60 S/T‡
1	Daily, Days 1–9	400	11.5	T§	0/10	8.0	T	0/10
(9.0)		200	29.0	222	4/10	10.5	T	0/10
		100	20.5	128	0/10	12.5	39	0/10
		50	16.0	77	0/10	18.5	106	0/10
		25	16.0	77	0/10	19.0	111	0/10
		12.5	15.0	67	0/10	18.0	100	0/10
		6.25	11.0	N§	0/10	16.0	78	0/10
		3.13	—	—	—	12.5	39	0/10
		1.56	—	—	—	11.0	N	0/10
2	Q3h/24h,	50	>64	>600	7/10	5.0	T	0/10
(9.0)	Days 1,5,9	25	25.5	183	0/10	7.5	T	0/10
		12.5	20.5	128	0/10	28.5	217	3/10
		6.25	17.0	89	0/10	>64.0	>600	8/10
		3.13	15.0	67	0/10	23.5	161	0/10
		1.57	11.0	N	0/10	19.5	117	0/10
		0.79	10.0	N	0/10	16.0	78	0/10
3	Once, Day 1 only	2000	4.0	T	0/10	13.0	44	0/10
(9.0)		1600	4.0	T	0/10	12.0	33	0/10
		800	14.0	56	0/10	10.0	N	0/10
		400	12.0	33	0/10	10.0	N	0/10
		200	11.5	28	0/10	10.0	N	0/10
		100	11.0	N	0/10	9.5	N	0/10

*Tumor inoculum = 10^5 cells/mouse. Untreated control median survival time (MST) = 9.0 days (no survivors).

†Percent increase in MST over controls.

‡No. of 60-day survivors/total treated.

§T = toxic (ILS < 25% at a dose > the OD).

N = inactive (ILS < 25% at a dose < the OD).

kemic activity typical for daily treatment with anhydro-ara-C. The difference in the daily OD of anhydro-ara-C solutions prepared fresh daily (400 mg/kg; five survivors/ten mice treated) and aged (200 mg/kg; four survivors/ten mice treated) was not greater than the previously observed differences between daily OD and activity in successive experiments (tables 1 and 2). Cytosine arabinoside solutions prepared fresh daily or aged failed to elicit a significant number of long-term survivors in the same experiment (table 6). The data suggest that over the course of the experiment, anhydro-ara-C in aged solutions did not undergo a rapid change to cytosine arabinoside.

A preliminary experiment showed that a variant of L1210 selected for resistance to cytosine arabinoside was insensitive to anhydro-ara-C. Comparison of activities against the parent-sensitive tumor and the resistant subline, L1210/ara-C/R (table 7), clearly confirmed the cross resistance to anhydro-ara-C. In the same experiment, the superiority of daily treatment with anhydro-ara-C over daily treatment with cytosine arabinoside against the parent, cytosine arabinoside-sensitive tumor was again observed.

DISCUSSION

The observation that daily treatment with anhydro-ara-C is superior to daily treatment with cytosine arabinoside and approximately equal to q3h/24h/q4d cytosine arabinoside treatment of L1210 mouse leukemia (table 8), suggests that the duration of effectiveness per injection is greater for anhydro-ara-C than for cytosine arabinoside. That daily treatment pro-

TABLE 3.—Comparative schedule dependency of oral treatment with anhydro-ara-C or cytosine arabinoside against L1210 leukemia*

Oral treatment schedule	Dose (mg/kg/injection)	Anhydro-ara-C			Cytosine arabinoside		
		MST (days)	ILS† (%)	Day 60 S/T‡	MST (days)	ILS† (%)	Day 60 S/T‡
Once, Day 1 only	3200	—	—	—	5.5	T§	0/10
	1600	14.0	55	0/10	14.0	55	0/10
	800	12.0	33	0/10	12.0	33	0/10
	400	11.0	N§	0/10	11.0	N	0/10
Q3h, Day 1 only	400	5.0	T	0/10	5.0	T	0/10
	200	13.5	50	1/10	6.0	T	0/10
	100	11.5	28	0/10	15.0	67	0/10
	50	13.0	44	0/10	14.5	61	0/10
	25	12.0	33	0/10	12.5	39	0/10
Once each day, Days 1,5,9	3200	5.0	T	0/10	5.5	T	0/10
	1600	20.0	122	0/10	18.0	100	0/10
	800	17.5	94	0/10	16.0	78	0/10
	400	16.5	83	0/10	14.0	55	0/10
	200	12.5	39	0/10			
	100	10.0	N	0/10			
Q3h/24h, Days 1,5,9	200	10.5	T	0/10	5.0	T	0/10
	100	18.0	100	0/10	9.0	T	0/10
	50	18.5	106	0/10	22.5	150	2/10
	25	16.0	78	0/10	8.5	N	0/10
	12.5	15.0	67	0/10	12.5	N	0/10
Daily, Days 1–9	800	8.5	T	1/10	—	—	—
	400	21.5	139	0/10	10.0	T	1/10
	200	17.0	88	0/10	13.0	44	0/10
	100	15.0	67	0/10	15.5	72	0/10
	50	13.5	50	0/10	16.0	78	0/10
	25	—	—	—	11.5	28	0/10

*Tumor inoculum = 10^5 cells implanted ip/mouse. Untreated control median survival time (MST) = 9.0 days (0/16 survivors).

†Percent increase in MST over controls.

‡No. of 60-day survivors/total treated.

§T = toxic (ILS < 25% at a dose > the OD).

N = inactive (ILS < 25% at a dose < the OD).

duced long-term survivors among mice with early tumor indicates that anhydro-ara-C given once each day is capable of providing active inhibitor concentrations and a duration of effect at the target cell sufficient to achieve total tumor cell destruction at sublethal doses. These observations are in agreement with those of Hoshi et al (10). Assuming that the schedule dependency of cytosine arabinoside is due, at least in part, to its relatively rapid inactivation in vivo, the results are consistent with these authors' findings (10) that the hydrochloride of anhydro-ara-C was not deaminated by mouse kidney pyrimidine nucleoside deaminase after 3 hours incubation at 37 C, whereas cytosine arabinoside hydrochloride was completely deaminated within 1 hour. Steuart and Burke (11) have reported substantial increases in human leukemic cell deaminase levels associated with the development of clinical resistance to cytosine arabinoside. Considering the apparent minimal degree of anhydro-ara-C deamination in mice, the present observations suggest a number of possibilities. It is possible that (*a*) anhydro-ara-C per se acts in a manner similar to cytosine arabinoside, (*b*) anhydro-ara-C is

TABLE 4.—Schedule dependency of anhydro-ara-C and cytosine arabinoside in the treatment of advanced L1210 leukemia*

Treatment route and schedule	Dose (mg/kg/injection)	Anhydro-ara-C MST (days)	Anhydro-ara-C ILS† (%)	Anhydro-ara-C Day 30 S/T‡	Cytosine arabinoside MST (days)	Cytosine arabinoside ILS† (%)	Cytosine arabinoside Day 30 S/T‡
Sc; once, Day 5 only	4800	—	—	—	14.0	40	0/8
	2400	15.0	50	0/8	13.0	30	0/8
	1200	14.0	40	0/8	12.0	N	0/8
	600	13.0	30	0/8	12.0	N	0/8
	300	12.0	N§	0/8	—	—	—
Sc; q3h/24h, Day 5 only	600	9.0	T§	0/8	—	—	—
	300	15.5	55	0/8	—	—	—
	150	15.0	50	0/8	9.0	0	T
	75	14.0	40	0/8	14.0	40	0/8
	37.5	13.0	30	0/8	14.0	40	0/8
	18.7	—	—	—	13.5	35	0/8
	9.4	—	—	—	13.5	35	1/8
Sc; once each day, Days 5,9,13	4800	—	—	—	16.0	60	0/8
	2400	13.0	30	0/8	21.0	110	0/8
	1200	19.5	95	0/8	17.5	75	0/8
	600	17.0	70	0/8	16.5	65	0/8
	300	15.0	50	0/8	16.0	60	0/8
	150	14.5	45	0/8	—	—	—
Sc; q3h/24h, Days 5,9, 13	150	21.0	110	0/8	—	—	—
	75	22.0	120	0/8	—	—	—
	37.5	20.5	105	0/8	10.5	T	0/8
	18.7	20.0	100	0/8	21.0	110	1/8
	9.4	19.0	90	0/8	20.0	100	0/8
	4.7	—	—	—	19.0	90	0/8
Sc; daily, Days 5–13	600	11.5	T	0/8			
	300	18.0	80	0/8			
	150	20.0	100	0/8	10	0	0/8
	75	19.0	90	0/8	14	40	0/8
	37.5	18.0	80	0/8	17.5	75	0/8
	18.7	—	—	—	19.0	90	0/8
	9.4	—	—	—	16.5	65	0/8
Oral; daily, Days 5–13	1200	10.0	T	0/8			
	600	15.5	55	0/8			
	300	19.5	95	0/8	11.5	T	0/8
	150	17.5	75	0/8	16.0	60	0/8
	75	18.0	80	0/8	14.0	40	0/8
	37.5	—	—	—	14.5	45	0/8
	18.7	—	—	—	13.5	35	0/8

*Tumor inoculum = 10^6 cells implanted sc/mouse. Untreated control median survival time (MST) = 10.0 days (0/16 survivors).

†Percent increase in MST over controls.

‡No. of 30-day survivors/total treated.

§T = toxic (ILS < 25% at a dose > the OD).

N = inactive (ILS < 25% at a dose < the OD).

TABLE 5.—Relative effectiveness in mice with ic implanted L1210 leukemia*

Compound	Ip treatment schedule	Dose range (mg/kg/injection)	OD	ILS† (%)	Day 46 S/T‡
BCNU	Once, Day 3 only	10–40	40	150	3/8
Anhydro-ara-C	Daily, Days 3–11	100–800	400	145	0/8
Cytosine arabinoside	Daily, Days 3–11	10–80	80	95	0/8

*Tumor inoculum = 3×10^5 cells implanted ic/mouse. Untreated control median survival time (MST) = 10 days (0/16 survivors).
†Percent increase in MST over controls at OD.
‡No. of 46-day survivors/total treated.

TABLE 6.—Comparison of freshly prepared and aged solutions of anhydro-ara-C and cytosine arabinoside for L1210 antileukemic effectiveness*

Compound	Ip daily dose (mg/kg)	Solutions prepared each day (Days 1–9) and injected immediately			Solutions prepared on Day 0 only and injected daily, Days 1–9		
		MST† (days)	ILS‡ (%)	Day 60 S/T§	MST† (days)	ILS‡ (%)	Day 60 S/T§
Anhydro-ara-C	800	4.5	T¶	0/10	5.0	T	0/10
	400	>60	>567	5/10	12.0	33	2/10
	200	25.0	178	0/10	26.5	194	4/10
	100	20.0	122	0/10	22.0	144	0/10
Cytosine arabinoside	100	11.0	T	0/10	11.0	T	0/10
	50	18.0	100	0/10	19.0	111	0/10
	25	17.0	89	1/10	17.0	89	0/10
	12.5	15.0	67	0/10	15.0	67	0/10
Untreated controls	—	9.0	—	0/10	—	—	—

*Tumor inoculum = 10^5 cells implanted ip/mouse.
†MST = median survival time.
‡Percent increase in MST over controls.
§No. of 60-day survivors/total treated.
¶T = toxic (ILS < 25% at a dose > the OD).

transformed to cytosine arabinoside in vivo, or (*c*) both.

As far as we are aware, the mechanism of antitumor action of anhydro-ara-C and the relationship of anhydro-ara-C metabolism to cytosine arabinoside metabolism are unknown. Thus, anhydro-ara-C should be studied extensively at the biochemical pharmacologic level. If anhydro-ara-C acts without transformation to cytosine arabinoside, the present observation of its lack of effectiveness against L1210/ara-C/R suggests that an increased deaminase level may not be the major mechanism of resistance of this tumor variant and that the mechanisms of action of cytosine arabinoside and anhydro-ara-C may be similar. If such is the case, it is possible that anhydro-ara-C is activated by deoxycytidine kinase as is cytosine arabinoside (20), and that deletion of deoxycytidine kinase is responsible for the resistance of L1210/ara-C/R. A variant of L1210 has been developed for resistance to anhydro-ara-C by Mead et al (personal communication). Cross-resistance studies with L1210/anhydro-ara-C/R are in progress and the measurement of deaminase and kinase levels in L1210/ara-C/R and L1210/anhydro-ara-C/R are planned.

If the activity of anhydro-ara-C is due to transformation to cytosine arabinoside in vivo, the process appears to be sufficiently slow in mice to permit the maintenance of maximally effective cytosine arabinoside levels when anhydro-ara-C is given once each day. In such a case, anhydro-ara-C might represent a soluble

TABLE 7.—Comparative sensitivity of L1210 leukemia and the cytosine arabinoside-resistant subline, L1210/ara-C/R*

Compound	Ip treatment schedule	Dose (mg/kg/injection)	Parent line of L1210			Subline, L1210/ara-C/R		
			MST† (days)	ILS‡ (%)	Day 30 S/T§	MST† (days)	ILS‡ (%)	Day 30 S/T§
Cytosine arabinoside	Daily, Days 1–9	160	9.5	T¶	0/8	—	—	—
		80	18.5	131	0/8	—	—	—
		40	17.0	113	0/8	10.0	N¶	0/8
		20	15.5	94	0/8	11.0	N	0/8
		10	14.5	80	0/8	11.0	N	0/8
Cytosine arabinoside	Q3h/24h, Days 1,5,9	40	9.0	T	0/8	9.5	T	0/8
		20	27.0	238	3/8	10.0	N	0/8
		10	22.5	180	2/8	10.5	N	0/8
		5.0	21.0	162	1/8	10.0	N	0/8
Anhydro-ara-C	Daily, Days 1–9	800	4.5	T	0/8	6.0	T	0/8
		400	10.5	31	0/8	8.5	T	0/8
		200	12.0	50	0/8	10.0	T	0/8
		100	25.0	213	2/8	10.0	N	0/8
		50	20.0	150	1/8	11.0	N	0/8
Anhydro-ara-C	Q3h/24h, Days 1,5,9	200	8.0	T	0/8	11.0	T	0/8
		100	>30	>275	6/8	9.0	N	0/8
		50	26.5	231	3/8	10.0	N	0/8
		25	27.0	237	3/8	11.0	N	0/8
		12.5	23.0	187	2/8	11.0	N	0/8
Untreated controls		—	8.0	—	0/16	11.0	—	0/16

*Tumor inoculum = 10^5 cells implanted ip/mouse (CDF_1 male mice).
†MST = median survival time.
‡Percent increase in MST over controls.
§No. of 30-day survivors/total treated.
¶T = toxic (ILS < 25% at a dose > the OD).
N = inactive (ILS < 25% at a dose < the OD).

TABLE 8.—Influence of the ip treatment schedule of anhydro-ara-C or cytosine arabinoside on long-term survival in mice with early ip implanted L1210 leukemia (pooled data from tables 1, 2, 6, and 7)

Treatment schedule	Long-term survivors at optimal dosage levels* (No. survivors/total treated)	
	Anhydro-ara-C	Cytosine arabinoside
Daily, Days 1–9	18/46 (39%)	0/46 (0)
Q3h/24h, Days 1,5,9	17/26 (65%)	12/26 (46%)

*No. of survivors/total number receiving the optimal dosage level based on increase in median survival time.

"reservoir" for cytosine arabinoside. Studies of in vivo levels of anhydro-ara-C and cytosine arabinoside after anhydro-ara-C injection into mice are also in progress (Mead et al, personal communication). In addition, it would be interesting to determine the influence of nucleoside deaminase inhibitors such as tetrahydrouridine (21) on the in vitro and in vivo effects of anhydro-ara-C and to compare the results with the reported effects of such inhibitors on cytosine arabinoside metabolism (22,23).

Anhydro-ara-C appears to be a prime candidate for comparative pharmacologic studies and clinical trials in patients whose refractoriness to cytosine arabinoside is associated with increased tumor deaminase levels (11) and in patients who have not previously received cytosine arabinoside.

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A Quick Reference Chart on Cross Resistance Between Anticancer Agents [1,2]

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When considering the concomitant or sequential use of drug combinations in the treatment of disseminated cancer, it is obviously important to bear in mind the problem of cross resistance. Because of the increasing number of available drugs with different structures and mechanisms of action it is becoming more difficult to recall the published data on cross resistance, lack of cross resistance, and therapeutic potentiation. The purpose of this brief report is to bring together such data in a quick reference chart. For more detail the reader is directed to the reviews (1–3) and original publications found in the Reference section.

The cross resistance chart was developed from studies based on:

(*a*) Data acquired from transplantable animal tumors which had been converted via manipulation procedures to a state where they appeared to be unsusceptible or resistant to a given drug A. Usually, but not always, this state was reached by serial passage of the tumor through animals treated with increasing doses of the drug. Once a significant level of resistance was achieved, it was usually retained without further exposure of the tumor to the drug.

(*b*) Comparison of the activities of drug B against both the parent tumor line and the line resistant to drug A. If drug B proved to be equally effective against both lines, it was concluded that the drug A-resistant tumor was not cross resistant to drug B. However, if the parent tumor consistently responded well to drug B and the drug A-resistant line responded very poorly or immeasurably, it was concluded that the drug A-resistant line was cross resistant to drug B. In some instances, biochemical studies have provided convincing evidence of the basic mechanism of specific resistance and cross resistance.

A notation of examples of clear-cut therapeutic potentiation between pairs of drugs is also included in the chart. *It is emphasized that the observations of potentiation were made on previously untreated tumors, not drug-resistant lines.* By therapeutic potentiation we mean that a combination consistently provided a better therapeutic response than could be obtained with optimal doses of either drug in the combination when used alone.

Twenty-seven agents, most of which are of clinical interest, have been included in the chart. The drugs have been grouped in the following "classes" (4):

SPS = S-phase specific with respect to cell killing.

SPS;SL = S-phase specific, but self-limiting because they also affect RNA and/or protein synthesis and may slow entry of some cells into S phase, thus sparing them during a given exposure.

CS = Cycle specific; phase not so clearly established at the biochemical level.

CPNS = Cycle-phase nonspecific; short exposure kills a much higher fraction of cells than are in any given cell cycle phase at the time of exposure. Most of these agents are cytotoxic

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QUICK REFERENCE CHART ON CROSS RESISTANCE BETWEEN ANTICANCER AGENTS

(and Combinations which Have Shown Therapeutic Potentiation In Vivo)

	Mammalian Cancer Cell Lines Resistant to: (Agents Tested → in vivo)	1. Ara-C	2. Hydroxyurea	3. Guanazole	4. Pyridine-2-CATS	5. Amethopterin	6. 6-MP	7. 6-Thioguanine	8. 8-Azaguanine	9. 6-Methyl MPR	10. 5-FU	11. Prednisone (or Cortisone)	12. Vincristine	13. Vinblastine	14. Actinomycin D	15. Daunomycin	16. Procarbazine	17. Nitrogen Mustard	18. P. A. Mustard	19. Cytoxan	20. TEM (or Thio TEPA)	21. Myleran (or Mannitol Myleran)	22. BCNU	23. CCNU	24. Methyl CCNU	25. DIC (45388)	26. BIC (82196)	27. L-Asparaginase	REFERENCES
	1. Ara-C		S	S	S	S	S	Ⓟ	S		S		S		S			S		Ⓟ			Ⓟ	Ⓟ		S		S	3,6,7,8,9,10,11,12,29
SPS	2. Hydroxyurea	S		+	+																			Ⓟ					7,12
SPS	3. Guanazole																												
SPS	4. Pyridine-2-CATS	S	+	+																									7
	5. Amethopterin	S	S				S	+	S	S	S	S		S	S	S		S	S	S	S		S			S		S	1,2,3,11,13,29
	6. 6-MP	S				S		+	+	Ⓟ	S			S	S			S		S	S					S			1,2,3,14,15,16,29
SPS, SL	7. 6-Thioguanine	Ⓟ				S	+		+		S_1+		S	S				S	S	S	S		S						1,2,3,8,9,12
SPS, SL	8. 8-Azaguanine		S			S	+	+			S		S	S						S	S								1,2
	9. 6-Methyl MPR	S					Ⓟ																						12,14,15,16
	10. 5-FU	S				S	S	S	S				S		S			S	S	S	S_1+		S						1,2,3
?	11. Prednisone (or Cortisone)											Ⓟ																	17,18
CS	12. Vincristine	S									Ⓟ			+															2,3,17,18
CS	13. Vinblastine	S										+			+														2,3
	14. Actinomycin D					S	S					S	+	+		+		S											19
	15. Daunomycin						Ⓟ								+														12,20
	16. Procarbazine																												
	17. Nitrogen Mustard																		+	+	+								2,3
	18. P. A. Mustard					S							S	S				+		+	+	+	+							2,21,22
	19. Cytoxan	Ⓟ				S_1+	S				S						S	+	+		+	+	Ⓟ,+		Ⓟ		+		2,3,12,23,24,29
CPNS	20. TEM (or Thio TEPA)					S					S							+		+		+							12,23
CPNS	21. Myleran (or Mannitol Myleran)																												
	22. BCNU *	Ⓟ	S			S	S			S										Ⓟ,+	+			+			+		25,26,27
	23. CCNU	Ⓟ																											10
	24. Methyl CCNU																			Ⓟ									28
	25. DIC (45388)	S		S		S	S									S		S		S			S	S			S		29
	26. BIC (82196)	S		S	S	S	S				S					S			S	S	S		+	+		+			29,30
?	27. L-Asparaginase	S				S														S									3,11

+ = Cross resistance. S = Not cross resistant; resistant line was as sensitive as or more sensitive than the parent tumor.

Ⓟ = Therapeutic potentiation demonstrated in vivo against previously untreated neoplasms, not drug-resistant lines.

Therapeutic potentiation may result from
(a) additive antitumor activity without additive host toxicity,
(b) prevention of or delay in the emergence of resistance,
(c) biochemical potentiation with some selectivity for neoplastic cells,
(d) differences in the tissue distribution of drugs of a combination.

* A line of plasmacytoma (Pla-1) selected for resistance to BCNU was cross resistant to cyclophosphamide and TEM; whereas a line of L1210 leukemia selected for resistance to BCNU was sensitive to cytoxan.

to resting tumor cells in vitro. Bruce et al (5), with good reason, have classed some of these agents (eg, cyclophosphamide [Cytoxan] and BCNU) as cycle specific, not cycle-stage specific, because they are much more cytotoxic to proliferating lymphoma cells than largely resting marrow stem cells. Some of these agents are known to react with or complex DNA and may kill cells as they attempt DNA replication before repair.

In certain instances, boxes have been used to enclose results obtained with drugs that have similar mechanisms of "activation" and/or mechanisms of action at the molecular level (eg, purine antagonists activated by the same pyrophosphorylase; inhibitors of nucleotide reductase; classical alkylating agents; actinomycin D and daunomycin, and possibly the vinca alkaloids). Such relationships are of theoretical as well as practical interest.

It is not intended to imply that the patterns of cross resistance or lack of cross resistance obtained to date and reviewed in the subject chart will hold for all tumors which are truly drug resistant in a biochemical sense. However, in instances where different tumors have been selected with marked resistance to a given drug, it is fair to say that similar patterns of cross resistance or lack of cross resistance usually have been observed. In addition, cell culture lines of animal or human tumor cells selected for resistance to specific drugs have shown similar cross-resistance patterns (in those instances where comparable studies have been carried out).

In animal tumor systems, drug failure can result in the absence of demonstrable drug resistance (eg, when tumor cells gain entry into the central nervous system or when maximum tolerated doses of a drug simply do not kill sensitive tumor cells as fast as they are being replaced in intervals between doses). In some instances such failure has been confused with true drug resistance which can be demonstrated by the passage of cells (from a neoplasm advancing in spite of treatment) to other animals, and a determination of the response to treatment with the same dosages of the same agent.

Finally, it would appear that the need for continual attention to the available knowledge regarding cross resistance between anticancer agents is increasing because of:

(*a*) The clear demonstrations of superiority of certain combinations of drugs in the treatment of disseminated human and animal cancers, and the certainty that additional combinations will be considered in future therapeutic trial design.

(*b*) The increasing percentages of complete remissions now being achieved by single drugs or combinations in the more rapidly growing human and animal cancers. In some instances these appear to be "cures"; in certain others they may represent "near cures" where very large numbers of cancer cells have been reduced to very small numbers which then repopulate to numbers recognized as a "recurrence." The latter situation is one which may be conducive to selection and overgrowth of resistant subpopulations.

(*c*) The present effort to increase cure rates of solid tumors with widespread metastasis by surgical or radiologic ablation of the major mass(es) and chemotherapeutic treatment aimed at eradication of residual microcolonies. As in (*b*), drug resistance may be limiting if the number of tumor cells left after surgical or radiation therapy is high.

(*d*) The continually increasing number of classes of anticancer agents which show no indication of cross resistance.

LIST OF COMPOUNDS

1. **Ara-C:** NSC-63878: cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride; arabinosylcytosine; cytosine arabinoside
2. **Hydroxyurea:** NSC-32065
3. **Guanazole:** NSC-1895; 1,2,4-triazolidine, 3,5-diimino-
4. **Pyridine-2-CATS:** NSC-729; pyridine-2-carboxaldehyde thiosemicarbazone
5. **Amethopterin:** NSC-740; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]-benzoyl]-; methotrexate
6. **6-MP:** NSC-755; purine-6-thiol, monohydrate; 6-mercaptopurine
7. **6-Thioguanine:** NSC-752; purine-6-thiol, 2-amino-

8. **8-Azaguanine:** NSC-749; 7*H*-*v*-triazolo[4,5-*d*]pyrimidin-7-one, 5-amino-1,6-dihydro-
9. **6-Methyl MPR:** NSC-40774; 9*H*-purine, 6-(methylthio)-9-β-D-ribofuranosyl-, dihydrate; 6-methylmercaptopurine riboside
10. **5-FU:** NSC-19893; 5-fluorouracil
11. **Prednisone:** NSC-10023; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-
    Cortisone: NSC-9703
12. **Vincristine:** NSC-67574; leurocristine, sulfate (1:1)
13. **Vinblastine:** NSC-49842; vincaleukoblastine, sulfate (1:1), monohydrate
14. **Actinomycin D:** NSC-3053
15. **Daunomycin:** NSC-82151
16. **Procarbazine:** NSC-77213; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride
17. **Nitrogen mustard:** NSC-762; diethylamine, 2,2′-dichloro-*N*-methyl-, hydrochloride
18. **P.A. mustard:** NSC-8806; alanine, 3-[*p*-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, L-; L-phenylalanine mustard; melphalan; L-sarcolysin
19. **Cytoxan:** NSC-26271; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate; cyclophosphamide
20. **TEM:** NSC-9706; *s*-triazine, 2,4,6-tris(1-aziridinyl)-; triethylenemelamine
    ThioTEPA: NSC-6396; phosphine sulfide, tris(1-aziridinyl)-
21. **Myleran:** NSC-750; 1,4-butanediol, dimethanesulfonate; busulfan
    Mannitol myleran: NSC-37538; mannitol, 1,6-dimethanesulfonate, D-
22. **BCNU:** NSC-409962; urea, 1,3-bis(2-chloroethyl)-1-nitroso-
23. **CCNU:** NSC-79037; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-
24. **Methyl-CCNU:** NSC-95441; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-
25. **DIC:** NSC-45388; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-
26. **BIC:** NSC-82196; imidazole-4-carboxamide, 5-[3,3-bis(2-chloroethyl)-1-triazeno]-
27. **L-Asparaginase:** NSC-109229

References to Cross Resistance Chart

	Neoplasms Used	*Reference*
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2.	Various	HUTCHISON, D. J. Studies on cross-resistance and collateral sensitivity (1962–1964). Cancer Res 25:1581–1595, 1965.
3.	Various	HUTCHISON, D. J., and SCHMID, F. A. Cross resistance and collateral sensitivity. *In* Drug Resistance and Selectivity: Biochemical and Cellular Basis. In press.
4.	L1210 leukemia	SKIPPER, H. E., SCHABEL, F. M., JR., MELLETT, L. B., ET AL. Implications of biochemical, cytokinetic, pharmacologic, and toxicologic relationships in the design of optimal therapeutic schedules. Cancer Chemother Rep 54:431–450, 1970.
5.	Transplanted AK lymphoma	BRUCE, W. R., MEEKER, B. E., and VALERIOTE, F. A. Comparison of the sensitivity of normal hematopoietic and transplanted lymphoma colony-forming cells to chemotherapeutic agents administered in vivo. J Natl Cancer Inst 37: 233–245, 1966.
6.	L1210 leukemia	WODINSKY, I., and KENSLER, C. J. Activity of selected compounds in subline of leukemia L1210 resistant to cytosine arabinoside (NSC-63878). Cancer Chemother Rep 43:1–3, 1964.
7.	L1210 leukemia	SCHABEL, F. M., JR., LASTER, W. R., JR., and TRADER, M. W. Specific DNA inhibitors vs leukemia L1210. Development of resistance to ara-C and ribonucleotide reductase inhibitors. Proc Am Assoc Cancer Res 12:67, 1971.

	Neoplasms Used	*Reference*
8.	L1210 leukemia	SCHMIDT, L. H., MONTGOMERY, J. A., LASTER, W. R., JR., ET AL. Combination therapy with arabinosylcytosine and thioguanine. Proc Am Assoc Cancer Res 11:70, 1970.
9.	Human acute myeloblastic leukemia	CLARKSON, B. D., and FRIED, J. Changing concepts of treatment in acute leukemia. Med Clin N Am 55:561–600, 1971.
10.	L1210 leukemia	SCHABEL, F. M., JR. In vivo leukemic cell kill kinetics and "curability" in experimental systems. *In* The Proliferation and Spread of Neoplastic Cells (21st Annual Symposium on Fundamental Cancer Research. The University of Texas M. D. Anderson Hospital and Tumor Institute). Baltimore, Williams and Wilkins Co., 1968, pp 379–408.
11.	L5178Y leukemia	SCHMID, F. A., and HUTCHISON, D. J. Induction and characteristics of resistance to L-asparaginase (NSC-109229) in mouse leukemia L5178Y. Cancer Chemother Rep 55:115–121, 1971.
12.	Murine leukemias	SCHABEL, F. M., JR., LASTER, W. R., JR., and TRADER, M. W. Therapeutic potentiation of antileukemic drugs. Proc Am Assoc Cancer Res 9:62, 1968. Schabel, F. M., Jr. Unpublished data.
13.	Murine leukemias	WODINSKY, I., and KENSLER, C. J. Activity of cytosine arabinoside (NSC-63878) in a spectrum of rodent tumors. Cancer Chemother Rep 47:65–68, 1965.
14.	L1210 leukemia, HEp 2 cells in culture	BENNETT, L. L., JR., BROCKMAN, R. W., SCHNEBLI, H. P., ET AL. Activity and mechanism of action of 6-methylthiopurine ribonucleoside in cancer cells resistant to 6-mercaptopurine. Nature 205:1276–1279, 1965.
15.	L1210 leukemia	SCHABEL, F. M., JR., LASTER, W. R., JR., and SKIPPER, H. E. Chemotherapy of leukemia L1210 by 6-mercaptopurine (NSC-755) in combination with 6-methylthiopurine ribonucleoside (NSC-40774). Cancer Chemother Rep 51:111–124, 1967.
16.	Acute myelogenous leukemia	HO, D. H. W., and FREI, E., III. Comparative biochemical and pharmacological studies of 6-methylthiopurine ribonucleoside (MMPR). Proc Am Assoc Cancer Res 9:31, 1968.
17.	Human acute lymphocytic leukemias	SELAWRY, O. S., HANANIAN, J., WOLMAN, I. J., ET AL. New treatment schedule with improved survival in childhood leukemia. JAMA 194: 75–81, 1965.
18.	Spontaneous AK leukemias	SKIPPER, H. E., SCHABEL, F. M., JR., TRADER, M. W., ET AL. Basic and therapeutic trial results obtained in the spontaneous AK leukemia (lymphoma) model—end of 1971. Cancer Chemother Rep 56:273–314, 1972.
19.	Cell culture (Chinese hamster cells)	BIEDLER, J. L., and RIEHM, H. Cellular resistance to actinomycin D in Chinese hamster cells in vitro: cross-resistance, radioautographic, and cytogenetic studies. Cancer Res 30: 1174–1184, 1970.

	Neoplasms Used	*Reference*
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21.	Walker carcinosarcoma 256, Dunning leukemia, Yoshida ascites hepatoma	SCHMIDT, L. H. Unpublished data.
22.	Walker carcinosarcoma 256, Yoshida ascites hepatoma	SCHMIDT, L. H. Experimental approaches to evaluating the activities of alkylating agents. Cancer Chemother Rep 16:25–28, 1962.
23.	Plasmacytoma 1 (hamster)	SKIPPER, H. E., and SCHABEL, F. M., JR. Experimental evaluation of potential anticancer agents. VII. Cross resistance of alkylating agent-resistant neoplasms. Cancer Chemother Rep 22:1–22, 1962.
24.	Walker carcinosarcoma 256	BOLLAG, W. Unpublished data.
25.	Transplanted AK lymphoma	VALERIOTE, F. A., BRUCE, W. R., and MEEKER, B. E. Synergistic action of cyclophosphamide and 1,3-bis-(2-chloroethyl)-1-nitrosourea on a transplanted murine lymphoma. J Natl Cancer Inst 40:935–944, 1968.
26.	L1210 leukemia	SCHABEL, F. M., JR. Improvement of therapeutic activity of antileukemia agents in experimental systems by varying dose schedules and by combination therapy. *In* Cancer Chemotherapy Dose Schedules and Combinations, Symposium B-10, 5th International Congress of Chemotherapy (Hall, T. C., and Karrer, K., eds). Vienna, Verlag der Wiener Medizinischen Akademie, 1968, pp 65–82.
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Combination Intermittent Chemotherapy for Patients With Disseminated Neuroblastoma [1,2]

Lawrence Helson,[3] Pharcharie Vanichayangkul,[4] Charlotte C. Tan,[3] Norma Wollner,[3] and M. Lois Murphy [3]

SUMMARY

The combined effects of surgical treatment and radiotherapy plus chemotherapy with vincristine, cyclophosphamide, and daunomycin in 20 patients with disseminated neuroblastoma were compared with previous results using only vincristine and cyclophosphamide during the course of chemotherapy. Moderate hematologic, neurologic, and gastrointestinal drug toxicity was well tolerated. Clinically beneficial responses occurred in 12 of 20 patients. In these 12 patients, the median duration of response was 17 months. The median survival time for patients diagnosed after 2 years of age was 18 months. Patients diagnosed before 2 years of age retained their potential for cure. Serial urinary vanilmandelic acid and catecholamine levels reflected the disease course in 85% of the patients.
[Cancer Chemother Rep 56:499–503, 1972]

In patients with disseminated neuroblastoma, the addition of cyclophosphamide (1) or vincristine (2) or both (3) in combination with surgical treatment and radiation therapy has not affected the cure rate but has increased the survival time.[5] In an attempt to further extend this therapeutic approach, a third agent, daunomycin (4–6),[6] was added to the chemotherapeutic regimen of 20 such patients.

MATERIALS AND METHODS

Between December 1968 and December 1970, 20 children with disseminated neuroblastoma were entered in this study (table 1). Clinical-pathologic staging was determined after tumor resection or biopsy (table 2). After surgical treatment, supervoltage radiation therapy was generally applied to the primary tumor site and to large or critically located metastatic deposits. Concomitant chemotherapy was given as long as the patient's peripheral white blood cell (wbc) count remained greater than 1500 cells/mm^3. One course of chemotherapy consisted of vincristine, cyclophosphamide, and daunomycin given over a 4–6-week period (table 3). Obligatory treatment-free intervals of 3–10 weeks separated courses (table 4).

Patients 10, 11, 17, and 20 had been treated with vincristine (4 mg/m^2 total dose) and cyclophosphamide (40 mg total dose) 1–3

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[5] Cyclophosphamide: NSC-26271; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl) amino]tetrahydro-, 2-oxide, monohydrate.

Vincristine: NSC-67574; leurocristine, sulfate (1:1).

[6] Daunomycin: NSC-82151. Supplied by the Cancer Therapy Evaluation Branch, Chemotherapy, NCI.

TABLE 1.—Response to surgical treatment, radiotherapy, and chemotherapy with the combination of vincristine, cyclophosphamide, and daunomycin in 20 patients with disseminated neuroblastoma

Patient No.	Age at diagnosis (mos)	Disease stage at diagnosis*	VMA and catecholamine levels at diagnosis†	Number of chemotherapy courses	VMA and catecholamine levels after therapy	Response category (duration)	Survival time of living patients (mos)	Current status‡	Survival time to death (mos)
1	2	V	A	3	N	I–C	34+	NED	
2	3	IV	A	2	A	0–0			16
3	8	IV	A	2	A	0–A (1 mo)			10
4	11	IV	A	2	N	I–C	25+	NED	
5	11	V	A	4	N	I–C	29+	NED	
6§	11	III	N	4	N	I–C	34+	NED	
7	12	IV	A	2	A	0–0			7
8	15	IV	A	3	N	I–A (12 mos)			16
9	22	IV	A	3	A	0–0			9
10	29	IV	A	3	A	0–A (6 mos)			23
11	47	IV	A	3	A	I–A (7 mos)			18
12	48	IV	A	3	A	I–A (4 mos)			7
13	52	IV	A	2	A	0–0			7
14	54	IV	N	4	N	I–A (16 mos)			24
15	60	IV	A	1	A	0–0			13
16	65	IV	N	3	A	I–A (18 mos)			19
17	85	IV	N	3	A	I–A (10 mos)			21
18	96	IV	N	3	A	I–A (10 mos)			14
19	154	IV	N	3	A	0–A (9 mos)			21
20	160	IV	N	4	N	I–A (18 mos)			38

*See table 2 for definitions.
†A = abnormal; N = normal.
‡NED = no evidence of disease.
§This patient had multiple disseminated intraperitoneal metastases.

TABLE 2.—Clinical-pathologic staging of neuroblastoma

Stage	Tumor	VMA and/or catecholamine levels*
I	Localized and completely excised.	N
II	Localized with contiguous extension to the same body side. Incompletely excised.	N
III	Regional metastases confined to the same body cavity and across midline.	A
IV	Distant metastases (eg, bone marrow with extrinsic cells). Roentgenograms show bony metastases.	A
V†	Liver or skin metastases. Patient <1 year of age.	A

*A = abnormal; N = normal.
†Modified after Pinkel et al (11) and Evans et al (12).

TABLE 3.—Course of combination drug therapy for metastatic neuroblastoma

Treatment day	Drug schedule
1	Vincristine (1.5 mg/m²) via rapid iv injection, followed by cyclophosphamide (1000 mg/m² or 40 mg/kg in 10 ml of water) via rapid iv injection, followed by electrolyte solution (400 ml/m² at 100 ml/hr).
8	Vincristine (2.0–2.5 mg/m²) via rapid iv injection.
15, 17, 19	Daunomycin (45 mg/m²) via rapid iv injection.
33	Vincristine (2.25 mg/m²) via rapid iv injection, followed by cyclophosphamide (1000 mg/m² or 40 mg/kg in 10 ml of water) via rapid iv injection, followed by electrolyte solution (400 ml/m² at 100 ml/hr).

months before this study. In addition, patient 20 received 75 μg/kg of actinomycin D (NSC-3053) 10 months before this study. Because of the small amounts of drug given over a short period of time, it was assumed that tumors in these patients probably would not have become resistant to these same agents.

Twenty-four-hour urinary excretion of vanilmandelic acid (VMA) and catecholamines was measured in all patients before therapy. Later, successive collections at daily, weekly, or greater intervals were taken. The values were compared with those from age-matched control patients. VMA excretion rates were determined by a method modified from Gitlow and Mahler (7,8). The normal upper limits were 120 mg of VMA/g of creatinine for children up to 12 months of age and 55 mg of VMA/g of creatinine for those over 12 months. Catecholamine excretion rates were determined by the method of Crout (9) with the upper limits of normal expressed as 0.19 mg of substance/g of creatinine for both age groups.

During the course of treatment patients were monitored for hematologic toxicity with determinations of hemoglobin levels and wbc and platelet counts twice weekly or more frequently if necessary. The extent of disease was recorded before and after each course of chemotherapy. Patients were surveyed for the presence of tumor in the bones and abdomen, with roentgenograms and intravenous pyelograms made every 2 and 4 months respectively during the first year of therapy. These procedures were repeated at longer intervals in patients responding to therapy. The responses and their durations were evaluated according to the Karnofsky response categories (table 5).

TABLE 4.—Schedule of drug administration according to disease stage

Treatment course	Rest period (wks)		
	Stage III	Stage IV	Stage V
1 }	6	3–4	6
2 }	8	3–4	8
3 }	10	3–4	8
4 }			

TABLE 5.—Evaluation according to Karnofsky response categories after administration of cancer chemotherapeutic agents (10)

Response category*	No. of patients	Duration of response (mos)
0–0	5	—
0–A	3	1,6,9
I–A	8	4,7,10,10,12,16,18,18
I–C	4	24,29,34,34

*Category 0

0–0: Disease progresses, no objective or subjective benefit.

0–A: Subjective benefit without favorable objective changes.

0–B: No subjective benefit but favorable objective changes.

0–C: Subjective benefit and favorable objective changes of less than 1 month's duration; disease then progresses.

Category I

I–A: Subjective benefit and objective changes in all measurable criteria (includes urinary VMA and catecholamine excretion rates) for 1 month or more.

I–B: Objective regression of all disease for 1 month or more in an asymptomatic patient.

I–C: Complete relief of symptoms, if any, and regression of all manifestations due to active disease for 1 year or more.

RESULTS

Clinically beneficial responses occurred in 12 of 20 patients. I–A responses (table 5) were assigned after objective regression of tumor and diminution, but not necessarily complete disappearance of, abnormal VMA and catecholamine excretion rates. For patients diagnosed after the age of 2, the median survival time was 18 months (range, 7–36 months). However, in some patients who ceased to respond to or did not respond to this therapeutic regimen, subsequent blood and platelet transfusions and administration of other chemotherapeutic agents appeared to have contributed to their overall survival times. In this study, four of nine children (44%) less than 2 years old at the time of diagnosis have been surviving for periods of 25–34 months.

Four of 13 patients with abnormal urinary VMA and catecholamine excretion levels at diagnosis responded to therapy with a decrease in tumor size and an accompanying gradual diminution to normal excretion levels within weeks to a year. Recurrent epidural tumor developed in one patient (No. 8) during the 12th month of therapy, and he died of cord and pulmonary metastases 4 months later without recurrence of abnormal urinary excretion. The other nine patients continued to excrete abnormal amounts until death. Progressive metastases developed in six of the seven patients who had normal levels at diagnosis, and in four, abnormal amounts of VMA and catecholamines were excreted paralleling tumor growth.

Gastrointestinal side effects of vincristine were noted in 25% of the patients. Constipation was generally avoided by the prophylactic administration of casanthranol and dioctyl sodium sulfosuccinate. No oral ulcers were observed. Cyclophosphamide-induced emesis was constant for all patients but could be moderated with concomitant administration of chlorpromazine. Among 114 cyclophosphamide administrations, hematuria occurred three times and was associated with inadequately hydrated patients. Alopecia developed in all patients by the end of the first course. Leukopenia was universal and generally reached its nadir after daunomycin administration, necessitating a 5–8-day delay before the administration of vincristine and cyclophosphamide. By prolonging the duration of a treatment course, the calculated dose could be given to all patients. Fourteen children received three or four courses over a period of 11–16 months as outpatients. None required hospitalization or a prolonged hospital stay because of chemotherapy toxicity.

The contribution of radiotherapy towards regression of the primary tumor was difficult to quantitate because of concomitant chemotherapy. Its use in the treatment of metastases involving the bones, orbits, chest, and soft tissues was appreciable in terms of palliation of symptoms and control of tumor growth. The slope of the fitted line in figure 1 indicates that there was no significant effect upon the length of survival time after the administration of cumulative doses greater than 2000 rads. Patients with progressive unremitting metastatic disease required frequent treatments and accumulated large doses within their relatively short survival time.

DISCUSSION

The addition of daunomycin to the drug combination of vincristine and cyclophosphamide produced an overall response rate of 60% and a median duration of response of 17 months. These results were similar to or better than those reported with cyclophosphamide alone (1), vincristine alone (2), these two drugs in combination (3), or daunomycin alone (4–6). Clinically beneficial responses occurred in 56% and 64% of the patients under 2 years of age and over 2 years of age at diagnosis

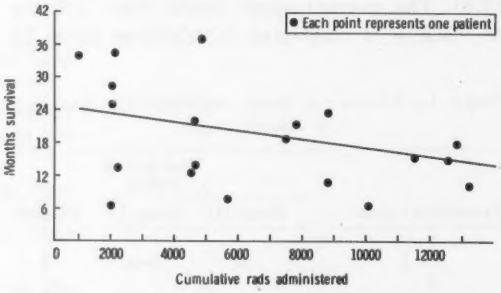

FIGURE 1.—Survival time and cumulative radiation administered for both curative and palliative therapy.

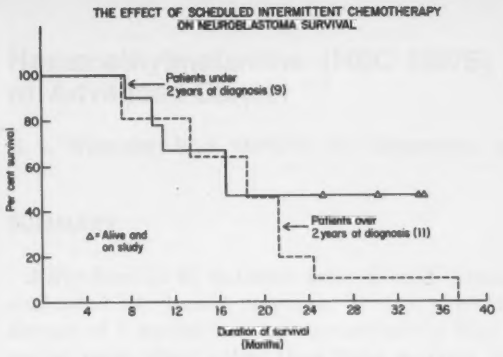

FIGURE 2.—Life table analysis of survival.

respectively. Such responses were long-lasting only in the patients under 2 years of age at diagnosis (fig 2).

When compared with a published median survival time of 8 months for combined vincristine and cyclophosphamide therapy (3), the addition of daunomycin prolonged the median survival time to 18 months for both age groups. Whether the increased duration of response and survival time can be used as a criterion for drug suitability can best be judged in the group of patients diagnosed at less than 2 years of age. These patients may be the most susceptible to improvement of their cure rate through treatment with effective drug regimens. This regimen did not produce any significant increase in cure rate in this group of patients and we cannot conclude that the addition of daunomycin was beneficial.

In this trial, the urinary VMA and catecholamine excretion rates reflected the disease status in 17 of 20 patients (85%). It was used in the original staging and in the evaluation of response to treatment. Eighty-one percent (13 of 16) of the patients with progressive disease had abnormal excretion values. Because of this it appeared justified to consider persistent abnormal values to be synonymous with active disease even if tumors were not otherwise clinically detectable.

These data suggest that the combination of vincristine, cyclophosphamide, and daunomycin administered intermittently to patients with disseminated neuroblastoma can increase survival time in the absence of debilitating toxicity. No improvement in cure rates was noted.

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Hexamethylmelamine (NSC-13875) in the Treatment of Advanced Cancer [1,2,3]

G. L. Wampler, S. J. Mellette, M. Kuperminc, and W. Regelson [4,5]

SUMMARY

Fifty-four of 97 patients treated with hexamethylmelamine were evaluated for tumor response. It was necessary to reduce the initial dosage of 8 mg/kg/day in approximately four of five patients. The major toxic effect other than bone marrow depression was nausea and vomiting, which occurred in 50% of the patients. Six of 30 patients with primary lung cancer responded to treatment. Five of these six patients had adenocarcinoma. Three of seven patients with ovarian carcinoma and one of eight with breast cancer responded also. There were only two complete remissions: one remission occurred in a patient with cancer of the ovary, and the other occurred in a patient with stage IV Hodgkin's disease. The remission of the latter patient has been maintained with intermittent maintenance therapy for more than 2 years and is of much better quality than that achieved previously with the use of the more conventional agents. The overall remission rate in patients for whom tumor response was evaluated was 20%. It is felt that continued study with particular attention to intermittent or combination chemotherapy is warranted.
[Cancer Chemother Rep 56:505–514, 1972]

Hexamethylmelamine, a congener of triethylenemelamine, was tested for antitumor activity in man. Unlike triethylenemelamine, hexamethylmelamine is not thought to act primarily as an alkylating agent (1); the mechanism of its action is not known. Hexamethylmelamine has been tested in phase II trials and has been found to have some activity against carcinoma of the lung, ovary, and breast, lymphoma, and to some extent, other tumors (2,3). A recent review of the literature concerning clinical studies with the drug was made by Carter and Livingston (4) and another review is in preparation.[6]

PATIENTS AND METHODS

Patients with advanced cancer not amenable to curative surgical treatment or radiotherapy and refractory to standard forms of chemotherapy, or patients for whom no satisfactory standard therapy was available were selected for the study. The drug was supplied in 50- and 100-mg capsules. According to the protocol, the initial dosage would be 8 mg/kg/day of hexamethylmelamine to be given orally in divided

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[2] This study was performed by the Eastern Cooperative Oncology Group and was supported by grant CA-10572 from the National Cancer Institute (NCI), National Institutes of Health.

[3] Hexamethylmelamine was supplied by the Cancer Therapy Evaluation Branch, Division of Cancer Treatment, NCI. Chemical nomenclature and NSC numbers for all compounds mentioned in this paper are listed in the List of Compounds at the end of the text.

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[5] The authors wish to thank Nina Stutts and Sandy Caldwell for data collection and assistance in preparing this manuscript.

[6] Blum, R., Livingston, R. B., and Carter, S. K. Clinical brochure: hexamethylmelamine (NSC-13875), Jan 1972. Prepared by the NCI, Bethesda, Md. In press.

doses. The dose was to be reduced to one half of the original dose if the white blood cell count (wbc) fell to less than 5000 cells/mm³ or if the platelet count fell to less than 150,000 platelets/mm³. Treatment was to be discontinued if the wbc fell to less than 3000 cells/mm³ or the platelet count fell to less than 90,000 platelets/mm³. There were occasional minor deviations from the protocol, but no patients have been eliminated from the study for this reason.

The records of 101 patients were reviewed for this study and, of these, only four were excluded from the study because it could not be established that they had received the medication after the initial dose. Patients were treated at the Medical College of Virginia Hospitals and Tumor Clinic and were evenly divided between private and staff physicians. Most patients were treated on an outpatient basis, but all had advanced cancer and, generally, a life expectancy of only several months. Only patients who were expected to live for at least 1 month were included.

Approximately two thirds of the patients had received prior chemotherapy; however, most (approximately 80%) of the patients with epidermoid carcinoma of the head and neck and many (approximately 50%) of the patients with lung cancer had not been treated previously. Almost 100% of the patients with cancer of the breast, cancer of the ovary, or lymphoma had received prior chemotherapy with typical combinations of cytotoxic agents over long periods of time. Many of the patients in the remaining disease categories had also received chemotherapy previously. Disease diagnoses are listed in table 1.

Forty-three of the 97 patients received treatment for less than 30 days; tumor response in these patients was not evaluated. Of these 43 patients, seven (16%) were excluded from the study because significant hematologic toxicity occurred. (One patient had a wbc of less than 3000 cells/mm³; two patients had platelet counts of less than 90,000 platelets/mm³; and four patients had both.) In this regard, all of these patients had bone marrow depressions resulting from previous chemotherapy at the time hexamethylmelamine treatment was started. Seven patients (16%) experienced severe or uncontrollable nausea and vomiting and were excluded from the study, and 11 patients (26%) died of their diseases within 1 month of the start of treatment. Nine additional patients (21%) were excluded because they deteriorated clinically or because they were unable to return to the clinic, and five patients (12%) were lost to followup study. For four patients, the records were incomplete.

For the patients who could tolerate more than 30 days of treatment, the occurrence of toxic effects was by itself not the major reason for discontinuing further treatment. Treatment was discontinued in approximately 8% of the patients because either hematologic, gastrointestinal, or suspected neurologic and systemic toxic effects occurred (one patient each). Most often, failure to control tumor growth at the tolerated dosage was the reason for stopping treatment. Several patients are still receiving the drug at the time of this writing.

Of the 54 patients treated for more than 30 days, dosage adjustments were required for 36 patients (78%). (Eight of the 54 patients received initial doses of less than 6 mg/kg/day and are not included in these percentage calculations.) Eight patients who received the prescribed initial dose of 8 mg/kg/day were treated at this dose level throughout the study and two others who received 6 mg/kg/day initially also were treated at this starting dose level throughout the study. The reasons for the adjustment of initial dosage (reduction in nearly all cases to one half of the original dose) are shown in table 2.

Criteria for Response

For this study, a complete remission is defined as one in which all measurable or other evidence of disease disappears and the patient returns to a normal functional state. A partial remission is defined as one in which the product of two diameters of a lesion is decreased by 50% or more, or, if more than one lesion is present, the average decrease in measurable lesions is 50% or more as computed previously. If only one diameter of a lesion is measured, the square of the diameter must be decreased by 50% or more. Progression of disease is defined as growth of lesions in excess of 100% of the baseline value as computed previously, death, or progressive clinical deterioration as measured by blood cell counts, serum enzyme

TABLE 1.—Disease diagnoses of patients treated with hexamethylmelamine

Disease	No. of patients— Initially included in the study (101 total)	Treated (97 total)	Treated 21+ days (68 total)	Treated 30+ days (54 total)	Treated 100+ days (19 total)
Cancer of the lung					
Squamous cell	12	12	10	9	1
Oat cell	4	4	4	2	0
Adenocarcinoma	7	7	7	6	6
Undifferentiated	4	4	1	1	1
Other	7	7	3	2	0
Total	34	34	25	20	8
Epidermoid carcinoma of the head and neck					
Maxillary antrum	1	1	1	1	0
Tongue	2	2	1	1	0
Tonsil	1	1	1	1	0
Mouth and throat (multiple)	1	1	1	1	0
Oropharynx	1	0	0	0	0
Epiglottis and pyriform sinus	1	1	1	1	1
Larynx	5	4	3	2	0
Total	12	10	8	7	1
Carcinoma of the ovary	13	13	8	7	3
Carcinoma of the breast	15	15	10	8	1
Carcinoma of the uterus and cervix	4	4	3	3	1
Hodgkin's disease	5	4	3	2	1
Reticulum cell sarcoma	2	1	1	1	0
Carcinoma of the bladder	3	3	2	2	2
Carcinoma of the urethra	1	1	1	0	0
Carcinoma of the pancreas	1	1	1	1	1
Carcinoma of the rectum	1	1	1	1	0
Carcinoma of the stomach	1	1	0	0	0
Ewing's sarcoma	2	2	1	1	1
Rhabdomyosarcoma	1	1	1	0	0
Neuroblastoma	1	1	1	1	0
Primary site unknown	5	5	2	0	0

tests, and physical examination. The category "no change" includes those patients not falling into any of the previously described categories.

RESULTS

Toxicity

The major toxic effects observed in this study are stated in tabular form in table 3 and include wbc and platelet count depression, nausea and vomiting, weight loss, weakness, lethargy, anorexia, diarrhea, neurotoxicity, and cystitis.

Hematologic toxicity was relatively mild and, in the patients selected for this study, the advanced stage of disease and the associated marrow involvement probably contributed more to the few cases of severe hematologic depres-

TABLE 2.—Reasons for hexamethylmelamine dosage adjustments for patients treated 30+ days

Toxic effect	No. of patients who had adjustments/ No. of patients treated 30+ days*	Percent
Wbc depression alone	16/46	35
Wbc and platelet depression	3/46	7
Platelet depression alone	2/46	4
Wbc and platelet depression with nausea	1/46	2
Wbc depression with nausea	1/46	2
Nausea and vomiting	11/46	24
Not stated	2/46	4
Total	36/46	78

*Total does not include 8 patients who received initial doses of less than 6 mg/kg/day.

sion than did the action of hexamethylmelamine. Except in patients with terminal disease, recovery of the marrow occurred promptly (7–14 days) after withdrawal of the drug or reduction in the dosage.

Wbc depression occurred more rapidly (5–120 days; average, 26 days) than did platelet count depression (7–120 days; average, 32 days), and for this reason it was more likely to be a factor in treatment modification. The percentage of patients with depressions in wbc counts was higher than the percentage of patients with depressions in platelet counts in both treatment categories. Eleven patients had wbc counts of less than 3000 cells/mm^3, three had counts of less than 2000 cells/mm^3, and none had counts of less than 1350 cells/mm^3. Ten patients had platelet counts of less than 90,000 platelets/mm^3. The lowest platelet count was 17,000 platelets/mm^3. Several patients with low platelet counts at the start of therapy tolerated the drug poorly. Despite the significant marrow depression mentioned previously no drug-related deaths were seen.

Nausea and vomiting occurred almost immediately after treatment in some patients, and after an interval of 1–32 days (averaged median, 14 days) in others. Occasionally these effects were so severe that therapy had to be stopped. The nausea was often refractory to phenothiazine antiemetics, but usually responded promptly to dosage modification. If the drug was tolerated for 30 days or more, nausea was no longer a major reason for suspension of treatment but, as indicated in table 2, it was the sole factor or a contributing factor in treatment modification in 13 of 46 (28%) of the patients. Significant nausea occurred in approximately 50% of the patients.

Progressive weight loss was seen in some patients and often was associated with nausea, weakness, and anorexia. These symptoms are generally seen in patients with advanced cancer (such as those in the study); nevertheless, it was our impression that hexamethylmelamine treatment was a contributing factor in some patients. In the group of 43 patients treated for less than 30 days, 11 (48%) of the 23 patients for whom weight loss was recorded lost more than 5 lbs. Included in the group of 54 patients treated for more than 30 days were patients responding to treatment (a positive factor) and patients with more natural constitutional tumor effects (a negative factor), and again, 48% (23 of 48 patients) lost weight (usually more than 10 lbs). We do not have a control group which would allow a more precise analysis of the weight change data.

There were no other significant toxic effects. Diarrhea thought to be related to treatment occurred in only three patients. Of the two terminally ill patients thought to have neurologic toxic effects, one had proved cerebral metastases. It is of interest that three patients had cystitis that was thought to be drug-related. The cystitis subsided after treatment was discontinued.

Tumor Responses

Evaluations of tumor response are shown in table 4. Responses were observed primarily in patients with adenocarcinoma of the lung and in patients with carcinoma of the ovary. For both of these conditions, the treatment resulted in reasonably prolonged remissions in a relatively high percentage of cases. We also had one complete response in a patient with Hodgkin's disease and one partial response in a patient with carcinoma of the breast. If our criteria for partial remission had been based on tumor regressions of 25%, we would have had a response rate of 43% for epidermoid tumors of the head and neck. These responses did not last long, however, and they were of little clinical value.

TABLE 3.—Toxic effects of hexamethylmelamine treatment

Toxic effect	Occurrence of toxic effect in patients treated—					
	Less than 30 days (43 total)	Percent	More than 30 days (54 total)	Percent	Total (97)	Percent
Wbc depression	12/33	36	28/48	58	40/81	49
Wbc 3000–5000 cells/mm³	9		20		29	
Wbc < 3000 cells/mm³	3		8		11	
Leukopenia at start	3*		4*		7*	
Leukopenia did not occur	21/33		20/48		41/81	
No record	7*		2*		9*	
Platelet depression	4/29	14	23/52	44	27/81	33
Platelet count 90,000–150,000 platelets/mm³	2		15		17	
Platelet count < 90,000 platelets/mm³	2		8		10	
Platelet count < 150,000 platelets/mm³ at start	6*		0		6*	
Platelet count never < 150,000 platelets/mm³	25/29		29/52		54/81	
No record	8*		2*		10*	
Nausea and vomiting	15/28	54	26/52	50	41/80	51
Mild to moderate	9		25		34	
Severe	6		1		7	
No record	15*		2*		17*	
Weight loss	11/23	48	23/48	48	34/71	48
5–10 lbs	5		5		10	
> 10 lbs	6		18		24	
Unchanged (± 5 lbs)	12		20		32	
Weight gain	0		5		5	
No record	20*		6*		26*	
Diarrhea					3†	
Neurotoxicity					2‡	
Cystitis					3§	

*Not included in calculation of percentage.

†One patient had diarrhea before hexamethylmelamine treatment was started.

‡One patient had cerebral metastases. In neither patient could symptoms be definitely attributed to hexamethylmelamine treatment.

§Symptoms appeared to be related to hexamethylmelamine treatment.

The drug was also clinically beneficial in two of three patients with carcinoma of the bladder and in several other patients who received treatment for more than 100 days (*see* table 1). The patients with carcinoma of the lung who responded received treatment for 105–665 days (average, 298 days). The three patients with ovarian carcinoma who responded received treatment for 180–245 days (average, 207 days). The patient with stage IV Hodgkin's disease was in the study 790 days and is still being treated. The patient with carcinoma of the breast was treated for 198 days. The duration of the responses corresponds with these figures (*see* table 4 and Case Reports).

Case Reports

Patient No. 1.—A 64-year-old housewife was examined in August 1968 and a diagnosis of papillary cystadenocarcinoma of the ovary with metastasis to the peritoneum was made. She was treated for 14 months with cyclophosphamide followed by chlorambucil with some success, but she had a relapse during chlorambucil therapy.

When treatment with hexamethylmela-

TABLE 4.—Response of tumors in patients treated with hexamethylmelamine

Diagnosis	Evaluated cases (54 total)	Complete response (2 total)	Partial response (9 total)	No change (27 total)	Progression of disease (16 total)
Cancer of the lung					
Squamous cell	9	0	0	4	5
Oat cell	2	0	0	1	1
Adenocarcinoma	6	0	5*	1†	0
Undifferentiated	1	0	1	0	0
Other	2	0	0	1	1
Total	20	0	6	7	7
Epidermoid carcinoma of the head and neck	7	0	0	4‡	3
Carcinoma of the ovary	7	1	2§	2	2
Carcinoma of the breast	8	0	1¶	5	2
Carcinoma of the uterus and cervix	3	0	0	3**	0
Hodgkin's disease	2	1	0	1	0
Reticulum cell sarcoma	1	0	0	1	0
Carcinoma of the bladder	2	0	0	2††	0
Carcinoma of the pancreas	1	0	0	1‡‡	0
Carcinoma of the rectum	1	0	0	0	1
Ewing's sarcoma	1	0	0	1	0
Neuroblastoma	1	0	0	0	1

*Patients in the study 392, 105, 198, 197, and 228 days, respectively.

†Greater than 25% objective decrease in node mass. Roentgenogram of the lungs was unchanged after 3½ months.

‡Includes 3 patients with objective regressions of more than 25% but less than 50%.

§*See* case reports for patients Nos. 1 and 3.

¶Patient in the study 198 days.

**Unmeasurable disease in 2 patients but these patients were stable clinically for 163 and 90 days, respectively.

††One patient had unmeasurable disease. This patient received the drug for 159 days and subjectively improved for more than 4 months. The other patient, treated for 150 days, had a regression in measurable disease of more than 25% but less than 50%.

‡‡Patient in the study 124 days.

mine was begun, the patient's abdominal circumference was 98 cm, her weight was 174 lbs, an ill-defined mass was present in the region of the bladder, and several small nodules were present high in the vagina. Treatment was started at a dose level of 8 mg/kg/day. Within 2 weeks, the abdominal circumference had decreased by 9 cm, and the patient's weight had stabilized at approximately 171 lbs. After 1 month, the soreness in the lower abdomen began to decrease and the patient reported that she was stronger and able to resume housework. During the following 4 months, the abdominal circumference increased but not to the original degree. After 7 months, a new nodule was noted and thereafter the ascites increased. After a total of 245 days, hexamethylmelamine treatment was stopped. The patient's drug dosage had been modified a number of times due to wbc depression and, for the last month, she was receiving only one fourth of the original dose.

Patient No. 2.—A diagnosis of Hodgkin's disease (granuloma type) was made in July 1965 for a 38-year-old housewife. There were multiple nodes in the axillary, cervical, and supraclavicular areas, and mediastinal widening which had been treated with "mantle" field radiation.

In early 1966, hilar and parenchymal lung disease with pleural effusion developed. Systemic chemotherapy was begun with chlorambucil initially and then with vinblastine; the therapy was successful and good response continued 20½ months. (All pulmonary disease responded.) Paraparesis secondary to epidural Hodgkin's disease in the D–10 area developed but successful laminectomy, additional radiation, and treatment with dexamethasone resulted in the complete return of all functions. Subsequently, paraspinal, lateral, and posterior chest wall masses developed but they responded to treatment with cyclophosphamide, vincristine, and procarbazine.

After the failure of procarbazine treatment, the chest wall masses rapidly progressed and for this reason hexamethylmelamine treatment was begun in August 1969. At that time, approximately six posterior chest wall masses 1.5–3.0 cm in size were present. Because the wbc had been affected, an initial dose of 300 mg/day (100 mg, three times a day; 6 mg/kg/day) was given. One week later this dose was raised to 400 mg/day (8 mg/kg) and, at the end of the second week, the chest wall masses were barely palpable. The 8-mg/kg dose could not be continued, however, after the first 3 weeks because thrombocytopenia (30.000 platelets/mm³) and leukocytopenia (wbc of less than 3000 cells/mm³) occurred. When doses of 2–4 mg/kg/day were given, the counts were satisfactory, but the disease recurred. After attempts to titrate the dosage with continuous therapy had continued for 5 months, it was found that the disease could generally be kept under satisfactory control and adequate counts could be maintained if a dose of 8 mg/kg was given daily for 7 days, and treatment was withheld the following 7 days.

After a year of this therapy, an inguinal node appeared but it regressed spontaneously with continued intermittent treatment. After 18 months of treatment, minimal pleural effusion occurred; this cleared without treatment modification.

After 2 years of treatment with hexamethylmelamine, the patient, who had been remarkably well and active during all this time, began to complain of malaise and significant increase of a posterior chest wall mass. An increase in the dose to 600 mg/day (about 10 mg/kg) for 1 week resulted in a greater than 50% reduction in the size of this mass and general improvement.

Currently, the patient is in continued remission even after 26 months of treatment with hexamethylmelamine, and continued intermittent treatment at a higher dose is planned. Her wbc and platelet counts have remained within the satisfactory range for the past 2 years, although the platelet count occasionally falls to 80,000–90.000 platelets/mm³. The only other toxic effect has been slight nausea which recurs with most courses of treatment but which is controlled by prochlorperazine.

Hexamethylmelamine has been the most satisfactory agent in the treatment of this patient with stage IV Hodgkin's disease. Her disease has been controlled now for almost 6 years. More than 2 years of this survival time can be ascribed to hexamethylmelamine treatment.

Patient No. 3.—A 43-year-old housewife with papillary cystadenocarcinoma of the ovary responded to chlorambucil treatment for 1 year but then the development of tumor ascites became evident. She was treated with 5-fluorouracil (5-FU) but did not respond, and palliative colostomy for large bowel obstruction was required in the winter of 1969. After this surgical treatment, she had recurrent episodes of partial obstruction with increasing ascites, general malaise, and pleural effusion in the left side of the chest. In April of 1969, hexamethylmelamine treatment was started at a total dose of 350 mg/day (6 mg/kg/day). Within the first week, there was evidence of a decrease in abdominal distention and the dose was increased from 350 to 400 mg/day, and within 2 weeks, the effusion in the left side of the chest disappeared. Because of the persistence of general weakness, prednisone (5 mg, three times a day) was added to the treatment regimen. By the fourth week of treatment, the patient's weight was stable, and there was a striking decrease in the amount of ascites and in all palpable disease as determined by the disappearance of a tumor in the rectum. By the fifth week, the nausea and vomiting had stopped, and the patient was able to eat small satisfying meals throughout the day. Her hexamethylmelamine dosage had to be decreased by the sixth week because of a fall in the wbc to 1750 cells/mm³ and a fall in the platelet count to 44,000 platelets/mm³.

After marrow recovery and 1 treatment-free week, the patient received hexamethylmelamine at a dose of 100 mg/day, which was subsequently increased to 200 mg/day; however, by the end of the second month of treatment, there was evidence of metastasis to her breast and chest wall. By the tenth week, pelvic disease reappeared and treatment was discontinued. The patient was given a brief course of treatment with phenesterin, but she died 2 months after the discontinuation of hexamethylmelamine treatment.

Patient No. 4.—A 62-year-old insurance salesman with a long history of regional enteritis was found to have adenocarcinoma of the lung with pleural effusion on the left side. The disease may have been present 2 years earlier since two small nodules were present in the upper lobe of the left lung (observed retrospectively). Treatment with 5-FU was started but the patient did not respond. Intrapleural quinacrine hydrochloride (Atabrine) instillation was not tolerated. The patient was treated with 300 mg/day of hexamethylmelamine, beginning in May 1969. Within 1 month, the pleural effusion dried up and there was subjective improvement in the patient's dyspnea. He was able to tolerate up to 500 mg/day (4 mg/kg) of hexamethylmelamine. Prednisone treatment was begun in an effort to improve his general strength and appetite; he was given this maintenance treatment until December 1969, when there was again evidence of progressive infiltration in the left side of the chest. Subsequently, the patient was treated with methotrexate, phenesterin, cyclophosphamide, and vincristine; however, he died of extensive lymphangitic tumor involvement 14 months after the start of hexamethylmelamine chemotherapy.

Patient No. 5.—A 52-year-old social worker who had had recurrent ovarian papillary cystadenocarcinoma was first referred to us in 1968. Fifteen years prior to her current tumor and following a cesarean section for the delivery of her second child, carcinoma of the left breast developed; this was treated by radical mastectomy. This was followed by radiation to the ovaries for radiation sterilization. Twelve years later in April 1965, adenocarcinoma of the right ovary with metastasis to the peritoneum, abdominal wall, and omentum developed. She was treated with radiation and was symptom-free for 3 years; however, in May 1968, anemia and recurrent pelvic disease were found and treatment with chlorambucil was started with prompt regression of all disease. Within 4 months, pelvic and abdominal disease were recurrent. The patient then received a large dose of methotrexate and methotrexate maintenance treatment at a dose of 2.5 mg/kg twice a week was started. There was moderate improvement in her general condition, but by December 1968, anorexia and bloody vaginal discharge occurred. A second course of chlorambucil treatment was unsuccessful. In February, a bypass of a recto-vaginal fistula was necessary with a side-to-side anastomosis of the ileum and a transverse loop colostomy was performed because the abdominal tumor was extensive.

Postoperatively, she received 5-FU intravenously (iv) with minimal improvement; by April 16, ascites had occurred and a new tumor had developed in the epigastric area. Hexamethylmelamine treatment was started April 21 at a half-dose of 4 mg/kg/day. Within 1 week, there was evidence of softening of the abdomen, and by May 7 (2 weeks after hexamethylmelamine treatment was started), there was remarkable response with a decrease in the size of the mass in the upper abdomen, and full mobilization of the ascites.

By June, all disease was clinically nonapparent and the patient received maintenance treatment at a dose of 100 mg of hexamethylmelamine every other day (the wbc was in the range of 4100 cells/mm^3), and by July, she returned to full-time work. By September, however, there was evidence of a recto-vesicle fistula, and by October, the liver was significantly enlarged. The dosage was increased to 200 mg/day (maintenance) and then to 600 mg/day, but by the latter part of October it became apparent that there was significant progression of the disease involving her liver. On December 21, 1969, treatment had to be discontinued. She was given a brief course of treatment with phenesterin from which she died 1 month later after massive breakdown of a metastatic mass in the right lung which produced a tension pneumothorax.

Patient No. 6.—A 41-year-old commercial fisherman and barge captain had tumor densi-

ties in his right lung in 1967 but he refused to enter a hospital until 1968 when a biopsy performed because of a pleural effusion on the right side revealed "undifferentiated carcinoma." When the patient was examined by us in July 1969, metastasis to his liver was extensive and the liver extended below the right iliac crest.

At the time that treatment was begun, it was felt that the patient would not survive more than 2 months because he had severe dyspnea and anorexia related to the disease in his lung and liver. Treatment with hexamethylmelamine was started at a dose of 600 mg/day; subsequently, this dose had to be reduced to a maintenance dose of 300–400 mg/day. The patient's liver mass gradually decreased in volume; this was accompanied by a decrease in the collateral cutaneous venous circulation in his abdomen. By November 1969 (4 months after treatment), he was able to return to work. By June 1970, he had a loss of general strength and treatment with prednisone (5 mg, four times a day) was started. Within 1 month he was once more able to return to full activity and he did well on this program until October 30, 1970, when tumor recurred on his thoracotomy scar. For this reason, vaccination with BCG (Bacillus Calmette Guérin vaccine) was attempted, but by November 25, 1970, there was another recurrence of the nodule in the skin of the thoracotomy scar. This disappeared after direct intratumoral inoculation with BCG, but by September 1971, there was evidence of bilateral metastasis of the disease to his neck and for this reason hexamethylmelamine treatment was discontinued after 25 months of relative control of the disease. The patient died of respiratory insufficiency 1 month later.

DISCUSSION

Our observations tend to confirm the findings of previous investigators (2,3), but these investigators have not shown the clear-cut difference between the effect of hexamethylmelamine treatment in patients with adenocarcinoma of the lung and the effect in patients with other types of lung cancer described in this report. Our population sample is small, however, and as our study progressed, there was a tendency to add patients with favorable diagnoses. This may have increased our percentage of responses (20% overall) which is comparable to that seen in published reports (2–4). Even though we have treated patients with advanced disease, the similarity between our results and those reported in the literature (ie, 16%, 19%, and 24% response rates) (2–4) indicates that our patient selection and experience are parallel to those of others. Better results could conceivably have been obtained with selection of patients with earlier, less advanced disease.

We conclude that hexamethylmelamine is a useful agent for the treatment of adenocarcinoma of the lung, carcinoma of the ovary, and Hodgkin's disease (lymphoma), not only because of the high percentage of response suggested by the results of our study, but also because of the relatively long duration of the remissions and the utility of the drug in patients with advanced cancer treated previously with other agents. We feel that more thorough testing is still required for carcinoma of the bladder and epidermoid carcinoma of the head and neck, cervix, uterus, and esophagus since the combined data to date suggest that hexamethylmelamine is active against these types of tumors even though only a small number of patients have been treated.

It is interesting that cystitis occurred in three patients treated with hexamethylmelamine; cystitis has occurred also in patients treated with cyclophosphamide.

Based on our experience with Hodgkin's disease, our feeling is that treatment at 2-week intervals is feasible although experimentation with dose and timing is in order since it may be that larger doses could be given at shorter intervals. This could reduce the occurrence of nausea and vomiting, which are serious dose-limiting toxic effects. Combination chemotherapy is also worth studying, particularly for the lymphomas and cancer of the breast or ovary. In any event, hexamethylmelamine is an active antitumor drug whose range of activity deserves further study.

LIST OF COMPOUNDS

Chlorambucil: NSC-3088; butyric acid, 4-[*p*-[bis(2-chloroethyl)amino]phenyl]-

Cyclophosphamide: NSC-26271; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate; Cytoxan

Dexamethasone: NSC-34521; pregna-1,4-diene-3,20-dione, 9-fluoro-11β, 17,21-trihydroxy-16α-methyl-

5-Fluorouracil: NSC-19893

Hexamethylmelamine: NSC-13875; *s*-triazine, 2,4,6-tris-(dimethylamino)-

Methotrexate: NSC-740; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-

Phenesterin: NSC-104469; cholesterol, [*p*-[bis(2-chloroethyl)amino]phenyl]-, acetate

Prednisone: NSC-10023; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-

Procarbazine: NSC-77213; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride

Prochlorperazine: NSC-17478; phenothiazine, 2-chloro-10-[3-(4-methyl-1-piperazinyl)propyl]-, dihydrobromide; Compazine

Triethylenemelamine: NSC-9706; *s*-triazine, 2,4,6-tris-(1-aziridinyl)-

Vinblastine: NSC-49842; vincaleukoblastine, sulfate (1:1), monohydrate

Vincristine: NSC-67574; leurocristine, sulfate (1:1)

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Phase I Clinical Trial of Weekly and Daily Treatment With Camptothecin (NSC-100880): Correlation With Preclinical Studies [1,2]

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SUMMARY

Tolerance to weekly and daily treatment with camptothecin was evaluated in patients with cancer. Fifteen patients were treated at four weekly dose levels ranging from 20 to 67 mg/m². Reversible leukopenia was the major dose-limiting toxic effect. Five-day loading courses were begun at total doses of 1.5 mg/m² per course because increased sensitivity to daily administration had been noted in animal studies. Leukopenia was more prolonged after daily treatment than after weekly treatment and occurred in four of six patients receiving a total dose of 100 mg/m². Tolerance to 5-day courses was an unexpected result. Also unpredicted by preclinical studies was man's susceptibility to cystitis with either schedule of treatment. Since the purpose of this phase I trial was to establish toxicity parameters, therapeutic response was not evaluable in all patients; however, two of ten patients in whom response could be evaluated had objective responses after weekly courses of treatment. No clinical benefit was observed in eight patients with measurable disease who were treated with the 5-day courses at dose levels associated with toxicity.

[Cancer Chemother Rep 56:515–521, 1972]

Camptothecin, an alkaloid from the stemwood of *Camptotheca acuminata* (1), is active against mouse L1210 leukemia (1), rat Walker carcinosarcoma (1), and several experimental tumors (2). It is a potent inhibitor of DNA synthesis, and in preclinical studies its antitumor properties and toxicity show marked schedule-dependence. The compound has recently been evaluated clinically by three groups of investigators (3–5). In the initial clinical trial (3), single or widely spaced doses of 2.5–10 mg/kg (approximately 100–400 mg/m²) per dose were used. Dose-limiting toxicity included myelosuppression and hemorrhagic cystitis (3). Because data from animal studies indicated that the effect of camptothecin against L1210 leukemia was schedule-dependent (table 1) and that toxicity occurred at much smaller doses of the drug when it was given daily as compared with intermittent ad-

[1] Received Dec 7, 1971; revised May 1, 1972.

[2] Camptothecin was supplied by Drug Research and Development, Chemotherapy, National Cancer Institute (NCI). Chemical nomenclature and NSC numbers for all compounds mentioned in this paper are listed in the List of Compounds at the end of the text.

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TABLE 1.—Camptothecin activity against L1210 leukemia*

Drug regimen†	Optimal dose (mg/kg/day)	% ILS‡	No. of surviving animals/ No. of leukemic animals tested	No. of surviving animals/ No. of normal animals tested
Day 1	30	55	0/10	8/8
Days 1–9	4 (total dose, 36)	53	0/10	7/8
Days 1, 5, 9	40 (total dose, 120)	115	0/10	7/8
Day 1; q3h	10	37	0/10	8/8
Days 1, 5, 9; q3h	10 (total dose, 30)	60	0/10	8/8

*Data from Drug Research and Development, Chemotherapy, NCI.
†Drug given intraperitoneally (ip) beginning on Day 1 after ip inoculation with 10^5 L1210 cells.
‡ILS = increased lifespan of treated vs control animals.

ministration, we began a clinical study to establish patient tolerance to weekly and daily dose schedules. Determination of a daily dose schedule would follow the establishment of a minimally toxic weekly dose.

MATERIALS AND METHODS

Patients

Patients with histologically confirmed cancer not amenable to conventional treatment were eligible for inclusion in the study provided their renal function was adequate as determined by a blood urea nitrogen (BUN) level of less than 25 mg/100 ml and a serum creatinine level of less than 1.5 mg/100 ml. Other requirements included absence of gross edema and hepatic decompensation, pretreatment white blood cell (wbc) and platelet counts in excess of 5000 cells/mm^3 and 100,000 cells/mm^3, respectively, and a sufficient interval after previous chemotherapy to avoid possible additive toxicity. Patients receiving nontoxic doses of camptothecin were eligible for a subsequent course at the next level after an interval of at least 2 weeks. At levels where toxicity was first encountered, previously untreated patients were preferentially treated; however, other patients could be treated at this level if 4 weeks had passed from the time of previous therapy. Of the 15 patients who received weekly treatment, four had not been treated previously, three had been exposed only to hormones and local radiotherapy, two had received cyclophosphamide with treatment discontinued 1 month prior to camptothecin therapy, and six had received courses of treatment with methotrexate, dibromodulcitol, or NSC-122819 (or a combination of two of these drugs) at least 2 weeks before the study. The cases of 17 patients who received daily treatment are discussed in this report. Eight of these patients had not been treated previously, five had been treated with local radiotherapy, and four had received courses of either 5-fluorouracil, methotrexate, bleomycin, or NSC-122819, 2–4 weeks prior to treatment with camptothecin.

The following tests were performed weekly during treatment and for at least 2 weeks after the completion of each treatment course: determination of wbc, platelet, and reticulocyte counts; urinalysis; determination of hematocrit value and levels of BUN, serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase, bilirubin, total serum proteins, and albumin. Clinical observations concerning gastrointestinal and urinary disturbances and other symptoms were made weekly. When tumors were present, they were measured weekly. Additional examinations such as skeletal and chest roentgenograms were obtained before the study. The chest roentgenograms were repeated every 2 weeks whenever measurable tumors were present; for patients without measurable tumors, roentgenographic examinations were repeated at the end of each study or when indicated clinically. Bone marrow aspirates were

obtained from two patients before and during the occurrence of drug toxicity.

Drug Administration

Camptothecin was supplied in vials containing 5 or 100 mg of the drug which was to be mixed in sterile water immediately before use, yielding a concentration of 1 or 10 mg/ml, respectively. The drug dissolved promptly and the resulting solution was slightly yellow. It was reported to be stable for at least 2 weeks when refrigerated, but the precipitation of fine crystals was observed when the solution was left standing. It was, therefore, always prepared just before treatment and administered by intravenous (iv) push. All courses were interrupted when leukopenia (defined as a fall in the wbc to less than 5000 cells/mm^3) or thrombocytopenia (defined as a fall in the platelet count to less than 100,000 cells/mm^3) or both occurred. Treatment was discontinued also when hematuria or other symptoms of cystitis occurred.

Drug Schedules

Weekly courses.—The drug was administered initially at a dose of 67 mg/m^2 which represented about one half of a tolerated toxic (single) dose for man reported by Gottlieb et al (3). At this weekly dose, the toxicity observed was tolerable but subsequent doses were decreased by one third to establish a minimally toxic weekly dose.

Loading courses (ie, daily for 5 days).—After tolerance to repeated weekly administration of 20–30 mg/m^2 had been established, daily administration was cautiously undertaken at a dose of 0.3 mg/m^2/day or a cumulative total dose of 1.5 mg/m^2. Doses were escalated after three patients had been treated at each dose level with no toxic effects. Doses were initially increased by 100% and 50% and subsequently by 33%.

RESULTS

Weekly Courses

Leukopenia and thrombocytopenia were the most common toxic effects observed, occurring with all four weekly dose regimens studied (table 2). As was expected, at the highest dose level, fewer doses were required to produce hematologic changes, and these changes were more pronounced and more consistently observed. The median time of occurrence of the wbc and platelet count nadirs was 7 days after the preceding nontoxic dose; in three instances, the nadir was observed on Day 14. By Day 21 after drug administration, all but one of these values had returned to normal. The granulocytes appeared to be more severely affected, since five instances of relative lymphocytosis were recorded.

Platelet count depression was generally less pronounced, and in no instance was it accompanied by bleeding. Rebound thrombocytosis was not infrequent; upon recovery from thrombocytopenia, two patients had platelet counts of more than 800,000 cells/mm^3. A decline in hematocrit value, ranging from 1% to 9% with a median of 5.5%, was recorded in all patients but one. Severe reticulocytopenia was also observed in nearly all patients (table 2). It usually occurred before the leukopenia; in some patients, it was the only evidence of drug effect on hematopoiesis. Rebound reticulocytosis was also observed.

TABLE 2.—Hematologic changes in patients treated with weekly courses of camptothecin

Dose (mg/m^2)	No. of patient courses	Wbc ($\times 10^3$ cells/mm^3)		Platelets ($\times 10^3$ cells/mm^3)		Reticulocytes (%)		No. of doses given when wbc < 5000 cells/mm^3 occurred	
		Median	Range	Median	Range	Median	Range	Median	Range
20	4	4.9	1.6–11.4	155	62–305	0.8	0.2–1.6	5	4–6
30	7	4.8	3.8–9.8	288	230–409	0.6	0.1–1.5	3	3–6
44	6	3.5	2.1–9.6	188	120–405	0.6	0.1–2.2	3	1–4
67	4	3.5	2.6–4.4	126	97–327	0.1	0.1–0.3	2	1–3

Cystitis was the most prominent non-hematologic toxic effect observed. Bladder toxicity was dose-limiting in three patients receiving doses of 30, 30, and 20 mg/m², respectively, and occurred coincidentally with hematologic toxicity in one patient and alopecia in another at doses of 44 and 30 mg/m². A clinical pattern similar to that for sterile cystitis preceded by complaints of urethritis and accompanied by hematuria was noted. Both the cystitis and the alopecia were completely reversible. Gastrointestinal toxicity was observed in only three of four patients receiving doses of 67 mg/m²; it was not observed at lower dose levels. It consisted of diarrhea in one patient, and nausea and vomiting in two others.

Disease diagnoses for patients in the study are shown in table 3. One patient with multiple subcutaneous nodules from an adenocarcinoma of the lung had a greater than 50% tumor regression lasting for 2 months. Subsequently, his condition improved and he experienced an objective response to methotrexate for 5 months. Another patient had a malignant gastric ulcer diagnosed by endoscopic brushing and deemed inoperable because the patient had cardiovascular disease. Complete healing of the ulcer occurred and there was no subsequent recurrence of the lesion. Disease progressed in all other patients.

TABLE 3.—Diagnoses of patients receiving camptothecin

Diagnosis	No. of patients receiving weekly courses	No. of patients receiving loading courses
Adenocarcinoma		
Prostate	3	—
Colon	2	1
Stomach	1	5
Pancreas	2	1
Lung	1	—
Primary site unknown	—	1
Epidermoid carcinoma		
Lung	2	—
Oropharynx	1	6
Esophagus	1	—
Primary site unknown	1	1
Malignant melanoma	1	—
Carcinoma of the bladder (transitional)	—	1
Hepatoma	—	1
Total	15	17

Loading Courses

Thirty-six treatment courses given at levels below total cumulative doses of 43 mg/m² were associated with no toxicity other than some diminution in hematocrit value, occasional reticulocytopenia, and in two patients receiving more than one such course, symptoms of cystitis. At necropsy, one of the patients with symptoms of cystitis was found to have squamous cell carcinoma of the bladder and adenocarcinoma of the prostate in addition to the previously known retroperitoneal leiomyosarcoma.

The hematologic changes observed at doses of 43 mg/m² and greater are shown in table 4. At a dose of 100 mg/m², leukopenia occurred in four of six patients. The granulocyte count continued to fall, and the nadir occurred 17–22 days after the patients had completed the course. In two of the patients, full recovery from leukopenia did not occur until 28 days after the last dose. Thrombocytopenia was marked only at the 100 mg/m² dose level, whereas reticulocytopenia was recorded in all instances. Reticulocytopenia occurred earlier than either leukopenia or thrombocytopenia. A drop in hematocrit value of at least 3% was recorded in all patients receiving 100 mg/m² and in two of six patients receiving 75 mg/m². One patient who received six daily doses to a total dose of 120 mg/m² also had a hematologic toxic response with a wbc nadir of 2800 cells/mm³ and a platelet nadir of 134,000 cells/mm³ both occurring on Day 10, and recovery occurring on Days 35 and 15, respectively, after the completion of treatment. Bone marrow was aspirated from the posterior iliac crests of two patients in whom leukopenia had occurred. No appreciable differences were noted in erythroid or myeloid morphology as compared to pretreatment marrow. Non-hematologic toxicity in these patients was confined to cystitis in three instances and alopecia in two. The cystitis occurred in two of the patients after total doses of 100 mg/m² had been given, and in one patient after a dose of 75 mg/m² had been given 4 weeks after a nontoxic course of 56 mg/m². Leukopenia occurred also in these three patients.

TABLE 4.—Hematologic changes in patients treated with camptothecin loading-course doses of 43 mg/m² or higher

Nadirs	Cumulative dose levels (No. of patient courses)				
	120 Mg/m² (1)	100 Mg/m² (6)	75 Mg/m² (6)	56 Mg/m² (3)	43 Mg/m² (4)
Wbc nadir (× 10³ cells/mm³)					
Median	2.8	2.9	5.9	6.5	5.5
Range	—	1.9–11.3	3.7–8.3	4.5–9.6	4.7–5.8
Medan time to nadir*	10	15	15	19	14
Granulocyte nadir (× 10³ cells/mm³)					
Median	1.40	2.40	3.65	6.10	3.90
Range	—	0.77–4.80	1.80–7.12	3.20–8.00	3.30–4.40
Median time to nadir*	22	18	17	—	—
Platelet nadir (× 10³ cells/mm³)					
Median	143	187	267	450	284
Range	—	62–560	225–395	267–660	230–508
Median time to nadir*	10	14	—	—	—
Reticulocyte nadir (%)					
Median	0.1	0.2	0.2	0.1	0.4
Range	—	0.1–0.4	0.1–0.4	0.1	0.1–0.9
Median time to nadir*	3	3	7	12	7

*Days from completion of course for patients with wbc < 5000 cells/mm³, granulocyte count < 2500 cells/mm³, platelet count $< 150{,}000$ cells/mm³, and reticulocyte count $< 1.0\%$.

The diagnoses of the diseases of the patients receiving the loading-course doses of 43 mg/m² or higher are shown in table 3 also. Eight of these patients had disease evaluable for therapeutic and toxic effects. None of the patients experienced tumor regression with this schedule of treatment.

DISCUSSION

In vitro studies with both normal lymphocyte cultures and L1210 cells indicate that camptothecin exerts a prompt potent inhibitory effect on the incorporation of thymidine (2,6,7). Inhibition of uridine incorporation can also be demonstrated (6–8); this effect on RNA synthesis is initially reversible and lags behind DNA inhibition (6,7). Cells in the synthetic phase of the cell cycle are primarily affected by camptothecin; toxicity to resting lymphocytes subsequently stimulated to divide has also been reported (2). This cell cycle stage-specificity of the drug may explain in part its schedule-dependent antitumor action and toxicity in preclinical studies. The current toxicologic data confirm the pronounced effects of camptothecin on renewing tissues, especially the erythroid and granulocytic elements. Gastrointestinal symptoms were not common at the dose levels investigated in the current study, but the gastrointestinal tract has been severely affected when higher doses of camptothecin are used (3,5). The latter effect was not unexpected, since hemorrhagic enterocolitis is a common toxic effect of camptothecin administration to animals; however, animals treated daily were clearly more susceptible than man to the gastrointestinal toxicity of camptothecin. Although toxicologic studies in dogs and monkeys are useful in predicting toxicity in man, such observations have certain limitations. The limitations of these observations for a number of other anticancer drugs have recently been reviewed (9).

Cystitis, another toxic effect occurring frequently after treatment with camptothecin, was not predicted by preclinical toxicologic studies. Our clinical experience would suggest that the occurrence of cystitis may be related to the duration of the patient's exposure to the drug. Camptothecin is excreted unchanged by the

kidneys, although a large percentage of the drug administered cannot be accounted for in the urine (3). It is possible that relatively less drug is excreted in the urine of animals since an extremely active transport of camptothecin into bile has been demonstrated (10). Alternatively, one needs to postulate that the mucosa of the human bladder is more susceptible to the toxic action of camptothecin or that the effect on the human bladder is due to some unrecognized camptothecin metabolite.

The finding that man's tolerance to daily courses of camptothecin far exceeds that of animals is noteworthy. Perhaps the marrow and the gastrointestinal tract of man can withstand the toxic effect of camptothecin longer, either because of the longer cell cycle times (compared to rodents) (11) or because man may have a higher threshold for susceptibility to the cytotoxic effects of the drug. The findings may also be explained by different patterns of drug excretion with less drug reaching the gastrointestinal tract in man. A third possibility is that we are able to influence renal excretion of camptothecin by vigorous hydration, thus allowing less accumulation of the drug in man as compared to animals. Evidence for such an effect on drug levels has been documented by measurements of the drug levels in the plasma prior to each daily dose (12); however, even patients who were not vigorously hydrated tolerated the daily courses better than would have been expected.

The therapeutic usefulness of camptothecin remains to be established. Encouraging objective responses in patients with adenocarcinoma of gastrointestinal origin after treatment with large intermittent doses of camptothecin were reported by Gottlieb et al (3); however, drug toxicity was severe. In a subsequent study by Moertel et al (5) confined to patients with advanced gastrointestinal cancer, only two of 32 patients receiving single doses of 120–180 mg/m² had partial responses (>50% reduction in tumor size) for more than 1 month, and no responses were seen with daily loading courses similar to ours. Response to treatment could be adequately assessed in only a small number of patients in this study: two responses were seen with the weekly schedule, and no responses were seen with the daily schedule. Future clinical studies of weekly treatment with initial doses of 44 mg/m² (in the absence of renal disease) and adjusted subsequent doses are feasible for other tumor types. The dose-limiting toxicity is likely to be hematologic, and the threat of gastrointestinal toxicity is minimized by this weekly schedule. However, cystitis will undoubtedly continue to pose problems in patients receiving prolonged treatment. On the other hand, a trial with daily treatment is not recommended because it is more likely to be associated with unpredictable drug accumulation leading to severe and prolonged hematologic toxicity.

LIST OF COMPOUNDS

Bleomycin: NSC-125066

Camptothecin: NSC-100880; sodium salt

Cyclophosphamide: NSC-26271; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

Dibromodulcitol: NSC-104800; galactitol, 1,6-dibromo-1,6-dideoxy-

5-Fluorouracil: NSC-19893

Methotrexate: NSC-740; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-

NSC-122819: epipodophyllotoxin, 4'-demethyl-9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside); VM-26; PTG

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5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388) in the Treatment of Advanced Acute Lymphocytic Leukemia in Children [1,2,3]

Jerry Z. Finklestein, Vincent Albo, Inta Ertel, Myron Karon, and Denman Hammond [4,5]

SUMMARY

5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388) was used to treat 20 children with advanced acute lymphocytic leukemia. Fourteen of the 20 patients received two or more courses of at least 200 mg/m²/day intravenously for 5 consecutive days. Nausea and vomiting were the main toxic effects. There were no objective responses in the 20 patients. Although treatment with the dose and schedule used in this study has been effective against other malignancies in man, it is not effective against advanced acute lymphocytic leukemia in children.

[Cancer Chemother Rep 56:523–526, 1972]

5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388) is a synthetic compound which is markedly effective against a variety of murine neoplasms.[6] Treatment with NSC-45388 and other triazenoimidazoles can cause a 90%–100% increase in the survival time of animals with L1210 murine leukemia and can also prolong the survival time of mice inoculated with Sarcoma 180 and Adenocarcinoma 775 (1–3).[6] Although its mechanism of action is unknown, NSC-45388 is not cell cycle specific (4). It appears to act either as an alkylating agent or as a purine antagonist (5,6).[7]

[1] Received Jan 5, 1972; accepted Feb 25, 1972.

[2] NSC-45388 was supplied by the Cancer Therapy Evaluation Branch, Chemotherapy, National Cancer Institute (NCI). Chemical nomenclature and NSC numbers for all compounds mentioned in this paper are listed in the List of Compounds at the end of the text.

[3] Supported by grants CA-07439 (Vincent Albo), CA-07336 (Wayne Borges), CA-11796 (Audrey E. Evans), CA-02649 (Myron Karon and Denman Hammond), CA-10382 (John R. Hartman and Ronald L. Chard), CA-02971 and CA-12047 (Ruth Heyn and Roxie Holland), CA-11996 and CA-07306 (William Krivit and Mark E. Nesbitt), CA-10198 (M. Eugene Lahey and John F. Wilson), and CA-11075 (L. Gilbert Thatcher) from the NCI, National Institutes of Health, and grant 4117 from the Ontario Cancer Treatment and Research Foundation (Marilyn Sonley).

[4] This study was done by the Children's Cancer Study Group A (Denman Hammond, chairman). The following investigators participated in the study: *Vincent Albo, William Prin,* and *Salvatore Orlando,* Children's Hospital of Pittsburgh, Pa; *Wayne Borges, Mila Pierce, James Nicklas,* and *Edda Hackl,* Children's Memorial Hospital, Chicago, Ill; *Inta Ertel,* Children's Hospital of Columbus, Ohio; *Audrey E. Evans, Milton H. Donaldson,* and *Hyder Carim,* Children's Hospital of Philadelphia, Pa; *Myron Karon, Denman Hammond, Jerry Z. Finklestein, Gerald Gilchrist, Gussie Higgins, Carol Hyman, Jorge Ortega, Nomie Shore,* and *Kenneth Williams,* Children's Hospital of Los Angeles, Calif; *John R. Hartman, Ronald L. Chard, Jr., Frank A. Reynolds, K. Fitzpatrick, I. Bernstein,* and *F. Johnson,* Children's Orthopedic Hospital, Seattle, Wash; *Ruth Heyn* and *Roxie Holland,* University of Michigan, Ann Arbor; *William Krivit* and *Mark E. Nesbitt,* University of Minnesota, Minneapolis; *M. Eugene Lahey* and *John F. Wilson,* University of Utah Medical Center, Salt Lake City; *Marilyn Sonley,* Princess Margaret Hospital, Toronto, Canada; *L. Gilbert Thatcher* and *Samuel R. McCreadie,* Milwaukee Children's Hospital, Wis.

[5] *Reprint requests to:* Jerry Z. Finklestein, MD, University of California, Los Angeles, Harbor General Hospital, 1000 West Carson St, Torrance, Calif 90509.

[6] Clinical brochure: imidazole carboxamide dimethyl triazeno (NSC-45388), Dec 1966. Prepared by the NCI, Bethesda, Md.

[7] Livingston, R. B., and Carter, S. K. Memorandum to the Scientific Director for Chemotherapy, NCI, Nov 1968.

In preliminary clinical trials, objective responses occurred in patients with malignant melanoma, fibrosarcoma, carcinoma of the lung, Hodgkin's disease, testicular tumors, cancer of the kidney, and various small cell sarcomas (6–8).[7] The purpose of this paper is to report the experience of Children's Cancer Study Group A with NSC-45388 in the treatment of 20 children with advanced acute lymphocytic leukemia.

MATERIALS AND METHODS

Children with acute lymphocytic or acute undifferentiated leukemia were eligible for inclusion in the study. With the exception of two patients who were treated with NSC-45388 before being treated with cytosine arabinoside, all patients were refractory to conventional antileukemic agents (prednisone, 6-mercaptopurine, methotrexate, cyclophosphamide, vincristine, and cytosine arabinoside). Seventeen children had received L-asparaginase and six had received daunomycin prior to the start of therapy with NSC-45388. For all patients, over 25% of the cells in marrow aspirates obtained at the start of therapy were lymphoblasts. All patients were considered to have status C or D disease (9). The patients ranged in age from 3 to 13 years.

NSC-45388 was mixed with 5% dextrose and water and infused intravenously (iv) over 15–20 minutes. This was done to avoid the pain which is experienced with rapid injection of the agent (6). The solution was administered immediately after reconstitution and was protected from light because of the compound's photosensitivity.

Patients received at least one course of 150–250 mg/m²/day iv for 5 consecutive days. Whenever possible, a second course of 150–400 mg/m²/day × 5 was administered 3–9 days later. Subsequent courses and doses were adjusted for each patient.

Physical examinations and hematologic evaluations were required at least once weekly and bone marrow aspirations were performed prior to the start of therapy and at monthly intervals when appropriate. Evaluation of response to therapy was made according to criteria previously published by Children's Cancer Study Group A (9).

RESULTS

Fourteen of the 20 patients received two or more courses of at least 200 mg/m²/day × 5 (table 1). One child (patient No. 14) received a total of five courses of treatment with NSC-45388 and tolerated up to 720 mg/m²/day × 5. Three patients received two or more courses of at least 150 mg/m²/day × 5. One child (patient No. 15) received two courses of 250 mg/m²/day × 5 days 20 days apart and a third course 14 days later, and two patients received only one course of 250 mg/m²/day × 5.

In all 20 patients, anemia (hgb<8 g/100 ml) and thrombocytopenia (platelet count<25,000 cells/mm³) occurred during therapy. Seventeen children received red blood cell (rbc) transfusions and 16 were given platelet concentrates. In 13 of the 20 patients, a decrease in total white blood cell count (wbc) was documented with the nadir occurring 2–19 days after the start of therapy (table 1).

Twelve of the 20 patients experienced nausea and vomiting secondary to the administration of NSC-45388. The nausea and vomiting usually could be controlled with antiemetics. Jaundice was documented in one child. Renal disorders and flu-like symptoms reported by other investigators (6) were not observed.

Leukemic cells did not disappear from the peripheral smears of the 17 patients who had had leukemic cells in the peripheral blood at the start of therapy. Leukemic cells occurred in the peripheral blood of one of the remaining three patients during treatment and marrow blasts increased from 25% to 95% in another. The third child had 100% lymphocytes in his peripheral blood throughout the course of therapy.

There were no objective responses. Of the 14 patients who received at least two courses of 200 mg/m²/day × 5, seven were removed from the study 19–55 days after the start of therapy. In the other patients, the disease progressed or death occurred 13–42 days after the start of drug therapy.

DISCUSSION

Various dosages of NSC-45388 have been used by other investigators; the schedule adopted in this study was slightly more ag-

TABLE 1.—Patient information

Patient No.	Sex	Age (yrs)	Duration of disease prior to treatment with NSC-45388 (mos)	Dose schedule		Wbc at Day 0 ($\times 10^3$ cells/mm^3)	Nadir wbc† ($\times 10^3$ cells/mm^3)	Day nadir occurred	Days in study	Reason for removal from study‡
				Mg/m^2/day	Day*					
1	M	13	30	250	0–4	1.2	0.8	19	27	PD
				400	14–18					
2	M	9	26	250	0–4	5.0	2.0	14	27	+
				200	15–19					
3	F	13	12	200	0–5	1.0	—	—	19	PD
				400	14–18					
4	F	3	23	225	0–4	120.0	93.0	12	15	+
				400	11–14					
5	M	12	18	250	0–4	13.1	10.5	6	19	+
				250	12–16					
6	M	3	22	250	0–4	84.0	16.4	16	16	+
				400	15–16					
7	M	5	38	250	0–4	5.4	3.6	14	28	PD
				400	14–18					
8	M	12	23	250	0–4	9.0	—	—	24	PD
				400	13–17					
9	M	5	26	250	0–4	20.1	—	—	21	PD
				400	14–18					
10	M	6	37	250	0–4	5.2	0.5	7	23	+
				250	14–18					
11	F	6	34	250	0–4	2.7	2.5	4	24	PD
				250	13–17					
12	F	12	28	250	0–4	2.0	0.8	6	26	+
				300	13–17					
13	F	4	26	250	0–4	8.8	—	—	22	+
				250	10–14					
				400	16–19					
14	M	7	46	250	0–4	6.6	0.7	5	55	PD
				360	12–16					
				360	21–24					
				720	28–31					
				720	37–41					
15	M	9	21	250	0–4	3.1	—	—	42	+
				250	28–30					
				250	42–44					
16	M	8	22	150	0–4	1.0	0.8	2	40	PD
				150	16–20					
				150	30–34					
17	F	4	56	150	0–3	7.5	1.0	3	23	PD
				150	8–11					
				250	12–14					
18	F	2	25	150	0–4	2.2	—	—	20	PD
				190	14–18					
19	M	6	19	250	0–4	3.8	—	—	13	PD
20	F	11	20	250	0–4	7.0	1.3	6	13	+

*First day of study = Day 0.
†Nadir wbc = lowest total white blood cell count.
‡PD = progressive disease and deterioration; + = death.

gressive than that reported by the Southwest Cancer Chemotherapy Study Group (150–250 mg/m²/day for 5 days with repeat courses given at 3-week intervals) (6–8). All of our patients tolerated the medication without undue difficulty and most patients received at least 250 mg/m²/day × 5. One patient tolerated up to 720 mg/m²/day × 5, and this suggests that higher doses may be tolerated in individual patients; however, preliminary evaluation of drug dosages in children with solid tumors who receive NSC-45388 indicates that 200–250 mg/m²/day × 5 every 14 days is probably an optimal dose. The decrease in total wbc seen in 13 children was probably an effect of the drug. It is not possible to consider the 100% incidence of thrombocytopenia and anemia as a manifestation of drug toxicity since these complications could be secondary to progressive disease. Phenothiazines usually were effective in alleviating the nausea and vomiting experienced by patients during treatment.

Gehan (10) has indicated that if 14 adequately treated patients fail to respond to a phase I trial of a new agent, there is less than a 5% chance that the response rate for the compound is 20%. Since the dose and schedule used in this trial were the same as or more aggressive than the dose and schedule used successfully in the therapy for other cancers (6–8),[8] one must conclude that NSC-45388 is inactive against advanced acute lymphocytic leukemia in children when used in the manner described in this paper.

LIST OF COMPOUNDS

L-Asparaginase: NSC-109229

Cyclophosphamide: NSC-26271; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro, 2-oxide, monohydrate

Cytosine arabinoside: NSC-63878; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride; ara-C

Daunomycin: NSC-82151

6-Mercaptopurine: NSC-755; purine-6-thiol, monohydrate; 6-MP

Methotrexate: NSC-740; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-; MTX

NSC-45388: 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide; DTIC

Prednisone: NSC-10023; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-

Vincristine: NSC-67574; leurocristine, sulfate (1:1)

[8] *See* footnote 7.

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Prediction of the Response of Patients With Acute Nonlymphocytic Leukemia to Cytosine Arabinoside (NSC-63878) Therapy [1,2,3]

C. Patrick Burns,[4] Steven A. Armentrout,[5] and Rune L. Stjernholm [6,7]

SUMMARY

Eighteen adults with acute nonlymphocytic leukemia received 22 courses of cytosine arabinoside therapy (4 mg/kg/day, administered as an 8-hour intravenous infusion) until bone marrow remission or intolerable toxicity occurred. Thymidine incorporation by the patients' leukocytes was measured in the presence of serum obtained before the first cytosine arabinoside infusion was started (control), and in serum obtained 2 hours after the first infusion was started. The clinical response of treated patients could be related to the degree to which the serum obtained during therapy inhibited thymidine incorporation. When the thymidine incorporation was depressed by 65% or more (compared with the control value), all patients had blast-free peripheral blood or bone marrow remission or both. In contrast, none of the patients in whom thymidine incorporation was depressed by 50% or less had a response of either kind. The absolute number of blast cells in the blood fell twice as rapidly in the group in which thymidine incorporation was depressed by 65% or more as in the group in which thymidine incorporation was depressed by 50% or less. In an intermediate group of patients (ten treatment courses) a depression in thymidine incorporation of 50%–65% was observed. The responses in this group were mixed. The collection of the sera into tubes containing tetrahydrouridine, a deaminase inhibitor added to prevent in vitro deamination of the drug, did not change the prediction of the response. Thus, the measurement of thymidine incorporation by leukocytes obtained from patients with acute nonlymphocytic leukemia and incubated with sera drawn before and during the first infusion of cytosine arabinoside provides a clue to in vivo cytotoxicity and induction of remission with this drug.
[Cancer Chemother Rep 56:527–534, 1972]

[1] Received Jan 17, 1972; revised May 19, 1972.

[2] Supported in part by grant CA-13214 from the National Cancer Institute, National Institutes of Health. A preliminary report appeared in abstract form (1).

[3] Cytosine arabinoside: cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride; ara-C. Supplied by The Upjohn Co., Kalamazoo, Mich.

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[7] Patients studied were at the University Hospitals of Cleveland, Cleveland Metropolitan General Hospital, St. Luke's Hospital, and Mount Sinai Hospital of Cleveland. We would like to thank the physicians from these institutions who aided in this regard.

Cytosine arabinoside is a nucleoside antimetabolite with known efficacy in the treatment of acute leukemia in adults. When the drug is used alone (2,3) [8] or in combination with other drugs (4–6), the probability of remission and lengthened survival time is increased. There is a great variation in the responses of patients with acute leukemia to treatment with the various chemotherapeutic agents. There are few methods available to the hematologist which enable him to predict which patients will respond to therapy with cytosine arabinoside and which patients should receive either unusually high doses of this drug or combinations of cytosine arabinoside and other chemotherapeutic agents.

Cytosine arabinoside prevents mitosis by inhibiting cellular DNA synthesis (7–9); however, there is considerable controversy about the mechanism of action of this drug and several theories have been offered (7,9–16). The action of cytosine arabinoside in inhibiting the DNA synthesis of leukemia cells in vitro may be related to the success of induction and maintenance of clinical remission. This study was undertaken in an attempt to relate the in vitro metabolic response of leukemic leukocytes to cytosine arabinoside to the clinical response observed subsequently. It was designed as an in vitro assay of the sensitivity of leukemic cells to cytosine arabinoside using drug-containing serum drawn during the early treatment period.

MATERIALS AND METHODS

Cytosine arabinoside was administered in an 8-hour intravenous infusion at a dose of 4 mg/kg/day. Treatment was continued until the absolute blast cell count per cubic millimeter was zero for 2 consecutive days, until the total white blood cell count (wbc) was less than 500 cells/mm^3, or until the patient failed to respond (indicated by a stable or rising absolute blast cell count for 4 consecutive days). Three patients (Nos. 1, 10, and 15) were studied during several different induction courses. A total of 22 courses of therapy administered to 18 patients was studied. The median number of treatment days for all courses was 7 days. No patient received less than 4 or more than 11 days of treatment with cytosine arabinoside. Sternal or iliac bone marrow aspirations were performed on patients who had blast-free peripheral blood after a course of therapy. The bone marrow aspirations were performed at a time when there was evidence from blood cell counts of recovery of the normal cell lines. A bone marrow biopsy with a Westerman-Jensen needle was performed when the aspirate specimen was inadequate or did not appear to reflect the true condition of the bone marrow. The criteria for remission were as follows: no blast cells in the peripheral blood, less than 5% blast cells in the bone marrow, and no evidence of leukemic disease in the parenchyma of organs or in the skin. A blast-free peripheral blood response was defined as one in which there were no blast cells in the peripheral blood but more than 5% in the bone marrow. If blast cells persisted in the peripheral blood, the induction attempt was considered a failure.

Leukemic cells for the metabolic studies were obtained immediately before the infusion of the cytosine arabinoside on the first day of the course. These cells were obtained from the peripheral blood for 20 courses, from the bone marrow for two courses, and from both sources in one case (patient No. 10, course No. 2). An anticoagulant (heparin) was added to the venous blood and the erythrocytes were sedimented by the addition of high molecular weight dextran. The leukocyte-rich supernatant was washed with phosphate-buffered saline and was suspended in Eagle's minimum essential medium (Grand Island Biological Co.). Viable cell counts were done with trypan blue.

For each determination, parallel sets of incubations were done. One set contained 20% autologous serum drawn immediately before the infusion, and the other contained 20% serum obtained after the first 2 hours of the infusion. DNA synthesis by leukocytes suspended in the serum obtained before the infusion served as a control. A method of handling the serum after venipuncture was designed to minimize and standardize the in vitro deamination of cytosine arabinoside. The blood was collected into glass test tubes and allowed to stand at 20 C for 5 minutes. Thereafter, it was maintained at 4 C and separated from the clot in a refrigerated centrifuge. Aliquots

[8] Armentrout, S. A., and Burns, C. P. Cytosine arabinoside therapy of acute granulocytic leukemia: a clinical evaluation. Submitted for publication.

of the blood drawn from five patients both before and during infusion were placed in glass tubes containing 100 μg of tetrahydrouridine [9] (supplied by Dr. Gary L. Neil, The Upjohn Co.) to give a concentration of 10 μg/ml of blood. Tetrahydrouridine inhibits the deamination of cytosine arabinoside (17).

Incubations with the radioactive substrates were carried out in 15 × 125-mm screwcap glass tubes containing 5 × 10^6 viable cells in 2 ml of culture medium. The tubes were flushed with a 95% air/5% CO_2 mixture for 8 seconds, closed, and placed in a roller drum at 37 C for 4 hours. Isolation of the nucleic acids by trichloroacetic acid precipitation has been described previously (18). Radioactivity was measured with a Tri-Carb liquid scintillation spectrometer (Packard Instruments Co.) and was corrected for quenching. Radioactive compounds were obtained from the New England Nuclear Corporation. The cells were incubated with 5 microcuries of tritiated thymidine (thymidine-C^3H_3) (14,700 dpm/picomol) for estimation of DNA synthesis and with 5 microcuries of tritiated uridine (uridine-5-^{3}H) (58,000 dpm/picomol) for estimation of RNA synthesis.

Results were expressed as picomols of nucleoside incorporated per hour of incubation per 10^6 total cells. Values for thymidine and uridine incorporation for cells incubated with sera drawn before (control) and during (experimental) the cytosine arabinoside infusion were compared. Glucose utilization, acetate utilization, and isoleucine incorporation were determined as previously described (19).

Daily wbc counts and differential cell counts were determined before and during cytosine arabinoside therapy. The wbc and absolute number of blast cells per cubic millimeter were plotted on semilogarithmic paper. The values for Days 1–10 after the first cytosine arabinoside infusion were used to calculate the rate of decrease in the wbc and blast cell counts. The data were evaluated by the "Student's t" test and by linear regression analysis.

Patient Selection

Eighteen adults with acute nonlymphocytic leukemia were seen at the University Hospitals of Cleveland and affiliated hospitals during the 11-month period from June 1970 through April 1971 (table 1). Tetrachrome stains of the peripheral blood and bone marrow cells formed the basis for the diagnosis and classification of acute leukemia. Cells from all patients were prepared for electron microscopic study. When lymphoblasts could not be excluded by morphologic criteria, peroxidase histochemical techniques (20) or determination of glucose utilization (21) were carried out. Five patients had received prior therapy for acute leukemia with radiation or drugs other than cytosine arabinoside. None had received any antileukemic therapy for at least 7 days before treatment with cytosine arabinoside. Two patients (Nos. 2 and 3) were in the blastic crisis of chronic myelocytic leukemia. Both had received busulfan [10] previously, but neither patient was taking the drug at the time of this study. Patient No. 4 had Turner's syndrome for which she had received cortisone acetate for many years. Results of liver function studies performed prior to the administration of cytosine arabinoside were normal for 15 patients and slightly abnormal for three. All patients were receiving allopurinol [11] (300 mg/day) at the time of the study.

RESULTS

Ten of the 22 courses of treatment produced complete remissions in seven patients for a remission rate of 45.5%. Five additional patients had blast-free peripheral blood after treatment. These patients are included in a larger clinical study reported elsewhere.[12]

The thymidine incorporation value in the control tubes ranged from 5 to 900 picomols/hr/10^6 cells compared with a thymidine incorporation value of 4–12 picomols/hr/10^6 cells for normal mature granulocytes and monocytes (Burns, C. P., unpublished data). Similar wide variations between the values for normal and leukemic cell thymidine incorporation have been noted in studies on the acute lymphoid leukemias (22,23). There was no relationship between the intensity of thymidine incorporation

[9] Tetrahydrouridine: NSC-112907; 2(1*H*)-pyrimidinone, tetrahydro-4-hydroxy-1-β-D-ribofuranosyl-; THU.

[10] Busulfan: NSC-750; 1,4-butanediol, dimethanesulfonate; Myleran.

[11] Allopurinol: NSC-108836; 1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ol, monosodium salt.

[12] *See* footnote 8.

TABLE 1.—Clinical and metabolic data on patients with acute

Patient No.	Course No.	Morphologic diagnosis*	Age, sex	Leukocyte count† ($\times 10^3$ cells/mm^3)	Blast cells‡ (%)
1	1	Myelocytic	21, F	32.1	85
	2	—	—	—	—
	3	—	—	—	—
2‡‡		Myelocytic	41, F	24.5	58
3‡‡		Myelocytic	41, F	82.5	74
4		Myelomonocytic	57, F	66.5	95
5		Myelocytic	29, M	6.8	36
6		Myelocytic	22, M	3.0	13
7		Myelomonocytic	56, F	130.0	82
8		Myelocytic	17, M	18.0	14
9		Myelomonocytic	86, M	24.5	3
10	1	Myelocytic	56, F	18.0	23
	2	—	—	—	—
11		Myelocytic	70, F	70.5	94
12		Myelomonocytic	43, F	175.0	98
13		Myelomonocytic	63, M	62.8	54
14		Myelomonocytic	59, F	1.2	2
15	1	Myelocytic	42, M	53.5	7
	2	—	—	—	—
16		Myelomonocytic	42, M	10.5	23
17		Myelomonocytic	67, M	6.0	18
18		Myelomonocytic	79, M	43.0	4

*Based on light and electron microscopy. All electron microscopic studies were performed by Dr. Richard Graham.

†Determined immediately before the first course of cytosine arabinoside treatment.

‡Percentage in the peripheral blood at the time of determination of leukocyte count.

§Spectrophotometric method (normal value, 5–7 μg/ml) (35).

¶From the time of diagnosis until the patient's death.

in control tubes and either the rate of fall in the peripheral blood blast cell count or clinical response. The intensity of the thymidine incorporation by the leukemic cells in the presence of serum drawn during the infusion did have some predictive value: all patients whose cells had thymidine incorporation rates of less than 35% of the control value had a response of some type (remissions occurred in six patients, and blast-free peripheral blood occurred in three) (fig 1). No patient whose cells had a thymidine incorporation rate of more than 50% of the control value achieved either response. Results in the intermediate group were mixed with complete response occurring in four patients, blast-free peripheral blood occurring in two, and treatment failure occurring in four.

In these same three groups, there was a stepwise difference in the rate of change in absolute blast cell count (table 2). The mean rate of decrease in blast cells for the group with thymidine incorporation values of less than 35% of the control values was approximately two times greater than that of the group with incorporation values of more than 50% of the control values ($P < 0.05$). There was no correlation between the percent reduction in thymidine incorporation and the rate of decrease in the total wbc.

Creatinine/blood urea nitrogen (mg/100 ml)	Serum lysozyme§ (μg/ml)	Duration of response (months)	Survival¶ (months)	Cause of death	Thymidine incorporation** (%)	Response††
—/< 18	7.7	2	—	—	9.9	CR
—	—	2	—	—	16.3	CR
—	—	1	12	Infection	29.2	CR
0.5/5	1.5	0.5	Alive	—	12.2	BF
1.0/13	18.2	0	6	Pulmonary embolus	18.6	BF
0.6/12	12.4	1	1	Infection	24.0	BF
0.8/13	11.0	3§§	Alive	—	26.7	CR
1.1/8	6.9	3§§	Alive	—	28.0	CR
1.3/25	67.0	0.25	6	CNS leukemia	38.7	CR
0.8/13	31.9	3§§	Alive	—	39.8	CR
2.4/90	100.0	0.5	3	Infection	40.4	BF
—/—	12.7	2	—	—	40.8	CR
—	—	0.5¶¶	4	Infection	24.3	CR
—/14	1.2	—	1	Infection	42.1	NR
0.9/8	101.3	—	1/6	Hemorrhage	43.0	NR
1.1/25	38.6	0.5	5	Hemorrhage	44.7	BF
0.8/13	15.0	—	Alive	—	45.2	NR
1.1/12	11.6	2.5	—	—	47.6	CR
—	—	—	10	Hemorrhage	43.4	NR
1.8/15	53.4	—	2	Infection	55.6	NR
—/17	11.1	—	1	Pulmonary leukemia	58.0	NR
—/23	42.0	—	Alive	—	81.1	NR

**Results are expressed as the percentage of the incorporation by control cultures of leukocytes in the presence of serum drawn prior to cytosine arabinoside infusion and are the mean values of duplicate determinations.
††CR = complete remission; BF = blast-free peripheral blood; NR = no response.
‡‡Blastic crisis of chronic myelocytic leukemia.
§§In remission at time of report.
¶¶Patient died while in remission.

TABLE 2.—Thymidine incorporation with sera obtained after infusion of cytosine arabinoside and rate of decrease of blast cells in the peripheral blood

Thymidine incorporation (% of control value)	Rate of decrease of blast cells*
> 50	−0.1544
35–50	−0.3046
< 35	−0.3147

*Expressed as the slope $\frac{\log Y_2 - \log Y_1}{X_2 - X_1}$ where Y = absolute blast cell count and X = days after the start of cytosine arabinoside therapy.

The results of the studies of thymidine incorporation using sera drawn into tubes containing tetrahydrouridine were analyzed separately. In five control experiments, tetrahydrouridine had no effect on leukemic cell incorporation of thymidine or uridine. There was no significant difference between the thymidine incorporation by leukemic cells incubated with serum drawn during the infusion and tetrahydrouridine and the incorporation by cells incubated without tetrahydrouridine ($P > 0.2$). This may indicate that the in vitro deamination of cytosine arabinoside was held to a minimum by the method of handling described, even in the absence of tetrahydrouridine. The predictive values of the studies with tetrahydrouridine were similar to those obtained without the inhibitor. All three patients experiencing

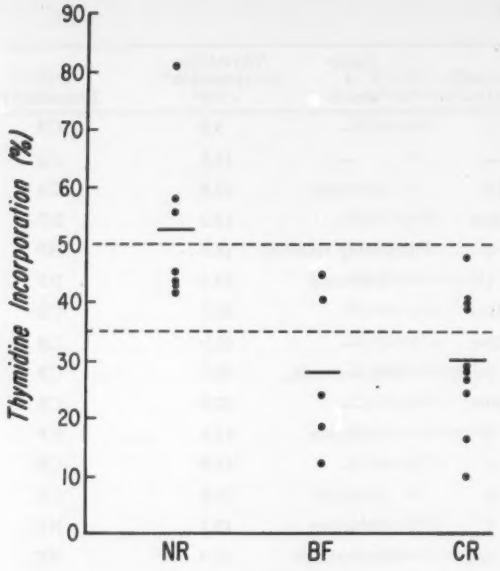

FIGURE 1.—Thymidine incorporation by leukemic cells in the presence of sera drawn during cytosine arabinoside infusion (grouped by clinical response). Values are expressed as percentages of control cultures (sera drawn before cytosine arabinoside infusion). Each point represents a course of therapy. Short horizontal bars are the means of the groups. NR = no response; BF = blast-free peripheral blood; and CR = complete remission.

some type of response (remissions in two patients and blast-free peripheral blood in the other) had thymidine incorporation values of less than 35% of the controls.

Uridine incorporation values with sera obtained before the infusion of cytosine arabinoside ranged from 6 to 135 picomols/hr/10^8 cells with a mean of 31.6 picomols as compared with values of 5–11 picomols/hr/10^8 cells for normal mature leukocytes. Isoleucine incorporation ranged from 12 to 33 nanomols/hr/10^8 cells with a mean of 20.8 nanomols compared with values of 4–5 nanomols/hr/10^8 cells for normal mature leukocytes (24). Neither changed significantly when the cells were incubated with cytosine arabinoside-containing sera.

Simultaneous studies of leukemic cells from both the peripheral blood and the bone marrow were performed for patient No. 10. The thymidine incorporation was depressed to 24% of the control value for the cells from the peripheral blood and to 11% for those from the bone marrow. Both values were well within the less-than-35% thymidine incorporation prognostic group and, therefore, were of similar predictive significance. The greater depression of thymidine incorporation in the bone marrow cultures probably reflects the higher percentage of blast cells it contained.

Thymidine incorporation by the cells of five patients was determined 4 days after the first study. No relationship could be established between the change in thymidine incorporation from Day 0 to Day 4 and the subsequent clinical response.

DISCUSSION

Previous attempts to predict clinical response to cytosine arabinoside in patients with acute leukemia based on a change in some metabolic function measured in vitro have had variable results. In several studies, a parallel between biochemical change and clinical response was demonstrated (10,25–27), but in other studies, such a correlation could not be demonstrated (11). In most instances, the tests were technically difficult, the number of patients was small, the clinical response was assessed only in general terms, and the therapy was not standardized. Often the metabolic parameter measured was not involved directly with the pathway affected by the drug. In many of the previous studies measuring isotope incorporation by leukemic cells in the presence of cytosine arabinoside, a labeled precursor less specific for DNA synthesis than thymidine (11,28–31) was used.

The system described in this paper offers the following advantages: (*a*) the leukemic cells are exposed to concentrations of the drug approximating those actually present in the individual patient's blood; (*b*) any variation in activation or inactivation of the drug will be reflected in the assay; (*c*) the actual site of cytosine arabinoside inhibition (DNA synthesis) is measured by thymidine incorporation; (*d*) the results can be evaluated by both the type and duration of therapeutic response and by the rate of decrease of the leukemic cells; (*e*) results can be obtained at the end of the first treatment day so that further therapy can be planned accordingly; (*f*) the procedure is

safe and relatively simple; (*g*) no enzyme assay is involved; and (*h*) the system offers an estimate of both in vivo cytosine arabinoside cytotoxicity and likelihood of remission.

The thymidine incorporation values for ten of the 22 treatment courses fell into the intermediate group. Results of the incorporation studies had no predictive value when the thymidine incorporation ranged from 35% to 50% (compared with controls). For patients with incorporation values within this range, the therapist must rely on the usual prognostic indicators. It is important to note that the intensity of initial thymidine incorporation by the cells incubated in autologous serum drawn before the start of therapy (control) was not correlated with the rate of fall of the blast cell counts during treatment or with the clinical response. The rates of RNA and protein synthesis failed to change after the cells were exposed to cytosine arabinoside in short term tissue culture. This demonstrates the futility of attempting to predict the responsiveness of leukemic cells to a drug using techniques designed for the evaluation of a metabolic process several steps away from that affected by the drug.

It is unclear whether differences in leukemic cell sensitivity to cytosine arabinoside are due to differences in the rate of deamination of the drug by the liver and wbc or to metabolic variability in the neoplastic cells. The former would be reflected in lower serum levels of cytosine arabinoside during the infusion. Since reliable biologic cytosine arabinoside assays are available, this question can be resolved (32). In one series of ten patients, there was a correlation between low plasma half-life and failure of cytosine arabinoside therapy (33). It has now been shown that the effectiveness of cytosine arabinoside in the treatment of acute leukemia is positively correlated with the rate of phosphorylation (activation) of the drug by deoxycytidine kinase (10,26,27). Moreover, leukocytes from patients responding to cytosine arabinoside therapy contain less cytidine deaminase (inactivation) than do leukocytes from nonresponding patients (34). Neither enzyme was measured in the present study. Our results indicate that this system, which does not require direct measurement of either the enzymatic activities mentioned previously or cytosine arabinoside levels in the blood, has definite predictive value.

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Intermittent Therapy With 6-Mercaptopurine (NSC-755) and Methotrexate (NSC-740) Given Intravenously to Adults With Acute Leukemia [1,2]

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SUMMARY

A randomized study of the effects of 6-mercaptopurine (6-MP) versus methotrexate given intravenously (iv) once or twice weekly was carried out in 159 adults with acute leukemia. Neither significant toxic effects nor therapeutic responses resulted from treatment once weekly with 800 mg/m² of 6-MP. Doses of 200–400 mg/m² of 6-MP given twice weekly did not regularly produce toxic effects, but 800 mg/m² given twice weekly did. Twice weekly doses of 6-MP of 1600 mg/m² were too toxic for practical use. The response rate with the various twice weekly doses of 6-MP did not vary significantly from rates previously obtained when the drug was given orally daily for either acute lymphocytic leukemia (ALL) or acute myelocytic leukemia (AML). Methotrexate given in doses of either 40–60 mg/m² weekly or 20–30 mg/m² twice weekly also did not produce toxic effects, but a dose of 40 mg/m² given twice weekly did result in toxicity; a dose of 60 mg/m² given twice weekly was too high. The response rate with methotrexate in the treatment of AML was slight, but was equal to that obtained when the drug is given orally daily. In ALL and acute unclassified leukemia in adults, the response rate to weekly or twice weekly iv therapy with methotrexate was higher than with daily oral therapy, producing two complete remissions and four partial remissions among 12 treated patients; the small sample size, however, limits interpretation of this finding. Despite the poor survival of the group as a whole, response to therapy is of importance to the specific patients involved, since occurrence of a remission is associated with an increased survival time.

[Cancer Chemother Rep 56:535–542, 1972]

[1] Received Feb 5, 1971; revised Mar 20, 1972.

[2] 6-Mercaptopurine: purine-6-thiol, monohydrate; 6-MP.

Methotrexate: glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-; Mtx.

6-Mercaptopurine and methotrexate were supplied by Drug Research and Development (formerly Cancer Chemotherapy National Service Center), Chemotherapy, National Cancer Institute (NCI).

[3] This study was done by the Acute Leukemia Cooperative Group B. The following institutions participated in the study: Roswell Park Memorial Institute, Buffalo, NY (grant CA-02599, *Drs. Ellison and Holland*); Mt. Sinai Hospital, New York, NY (CA-04457, *Dr. Hoogstraten*); Long Island Jewish Hospital, New Hyde Park, NY (CA-05923, *Dr. Levy*); Maimonides Hospital of Brooklyn, NY (CA-05923, *Dr. Lee*); New York Hospital, Cornell Medical Center, NY (CA-07963,

The treatment of acute leukemia with conventional daily oral doses of 6-mercaptopurine (6-MP) or methotrexate is far less effective in adults than in children. This is probably related only in part to the predominance of acute myelocytic leukemia (AML) in adults, since previous reports indicate little effect of methotrexate even in adults with acute lymphocytic leukemia (ALL) (1).[5]

In children with ALL, methotrexate given intravenously (iv) twice weekly maintains remissions induced by prednisone and vincristine [6] for a longer period than does methotrexate given in conventional daily oral doses (2,3), although an increase in the rate of remission induction is not produced with this iv methotrexate regimen (4).

This study by the Acute Leukemia Cooperative Group B (ALGB) tested methotrexate and 6-MP given iv once or twice weekly in adults with acute leukemia. Patients were admitted to the study from September 15, 1963, through January 8, 1965. Since that time, increasing attention has focused on schedule-related variations in the therapeutic index of new drugs which are useful in the treatment of acute leukemia. These data present several examples of schedule manipulation of the two antimetabolites originally used widely in the treatment of acute leukemia. In view of the current appreciation of the inadequacy of single-agent treatment of acute leukemia in adults. it is hoped that the data included herein will be of use in planning combination therapy.

METHODS

Two studies were undertaken. Patients were admitted to the first study (*a*) if their disease had been diagnosed as acute leukemia; (*b*) if their marrow contained 30% or more blast cells, promyelocytes, and/or other leukemic cells; (*c*) if they were 15 years of age or older; (*d*) if they had not received prior treatment with 6-MP or methotrexate for more than 2 weeks; or (*e*) if they had blood urea nitrogen (BUN) levels of less than 50 mg/100 ml. For the second study, the requirements were the same except that the BUN levels had to be less than 20 mg/100 ml.

Responses were evaluated by the then current ALGB modification [7] of the criteria de-

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[5] R. R. Ellison. Unpublished data from Memorial Hospital, New York, NY.

[6] Prednisone: NSC-10023; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-.

Vincristine: NSC-67574; leurocristine, sulfate (1:1).

[7] Complete remission required the following:

Marrow: No more than 5% leukemic cells and blast cells and no more than 15% leukemic cells, blast cells, and lymphocytes. The remaining marrow cells should be normal hematopoietic cells.

Peripheral blood: No leukemic cells or blast cells, hemoglobin level of 12 g/100 ml or greater (unrelated to transfusions), circulating granulocyte count of 2000 cells/mm^3 or greater, total leukocyte count not exceeding 15,000 cells/mm^3, and platelet count of 100,000 cells/mm^3 or greater.

Physical findings: No evidence of leukemic infiltration in any organ.

Symptoms: No symptoms attributable to leukemia.

Partial remission indicated that the criteria for complete remission were not satisfied in at least one category, but that in all categories the patients had at least the following:

Marrow: No more than 30% leukemic cells and blast cells and no more than 50% leukemic cells, blast cells, and lymphocytes.

Peripheral blood: No more than 20% leukemic cells and blast cells, hemoglobin level of 9 g/100 ml or greater (unrelated to transfusions), circulating granulocyte count of 1000 cells/mm^3 or greater, total leukocyte count not exceeding 20,000 cells/mm^3, and platelet count of 75,000 cells/mm^3 or greater.

Physical findings: Definite but not marked enlargement of lymph nodes, spleen, and liver.

Symptoms: More than 50% of normal activity with no more than mild symptoms, not requiring treatment.

vised by the Clinical Studies Panel of the Cancer Chemotherapy National Service Center (5). Only complete or partial remissions were considered to be of significant benefit. Patients in whom marrow and/or peripheral blood status returned to normal in the presence of progression or persistence of disease elsewhere (ie, node involvement, hepatosplenomegaly, etc) were not included among "remissions."

In study No. 1 (ALGB 6308), patients were randomized among four groups and received the following treatment: methotrexate at a dose of 20 mg/m² twice weekly or 40 mg/m² weekly, or 6-MP at a dose of 200 mg/m² twice weekly or 400 mg/m² weekly. Both drugs were given iv. Treatment was continued for a maximum of 70 days with a change to oral maintenance therapy as soon as complete remission values for the marrow and peripheral indications of remission or impending remission for the hematologic effects were achieved. If partial marrow remission developed, the iv therapy was continued until 70 days had elapsed; the patient then received oral maintenance drug. Patients were crossed over to the alternate drug after 70 days of treatment had elapsed without a response, after a relapse from response occurred, or if clinical or hematologic deterioration was occurring after 42 days of treatment with the initial drug.

Early in the study it became obvious that relatively few toxic side effects were occurring. The doses were therefore increased to 30 mg/m² twice weekly or 80 mg/m² weekly (methotrexate) and 400 mg/m² twice weekly or 800 mg/m² weekly (6-MP). Allowance was made for further escalation of the dose at 14-day intervals where indicated in an attempt to reach biologically meaningful dose levels as evidenced by marrow depression.

In addition, because of the low incidence of side effects, we started a second study (No. 2, ALGB 6411) with higher drug doses. The drugs were given twice weekly at the following doses: 6-MP, 800 or 1600 mg/m²; methotrexate, 40 or 60 mg/m². Both drugs were given iv. Early in this amended study, severe marrow depression or severe gastrointestinal toxicity or both were noted in most patients receiving the higher dose of either drug. These dose levels were therefore discontinued and randomization was continued between the lower doses of the two drugs.

Maintenance therapy in both studies consisted of administration of the drug which had produced remission: 5 mg of methotrexate or 3 mg/kg of 6-MP given orally daily.

RESULTS

Study No. 1

The cases of 90 patients were studied at the dose schedules specified. An additional ten patients were excluded from the study after randomization due to ineligibility or major deviations from the protocol. The 90 patients included 74 with AML and 16 with ALL or acute unclassified leukemia (AUL). Because patients with AUL resembled those with ALL more closely in remission behavior, they were analyzed as if they had ALL.

Twenty-four patients (20 with AML) received adrenal cortical steroids at some time during the drug trial. Four of these received steroids throughout the entire study and ten received steroids only at the end of the study. Two of the steroid-treated patients (both of whom were previously untreated) achieved remission, with the response probably due to the steroid, and these cases are, therefore, deleted from the data and further analysis is done on the remaining 88 patients only.

The results for the 88 patients are shown in tables 1 and 2. Five responses were obtained in 20 patients who received 6-MP twice weekly. Three of these responses were noted in the four patients with ALL and two in the 16 with AML. No remissions occurred when the drug was given weekly. It was also noted in the group receiving weekly therapy that any depression of the white blood cell count (wbc) was usually minimal or lasted less than 1 week or both.

The five patients who had marrow responses resulting from the twice weekly 6-MP regimen

TABLE 1.—Response in adults with acute myelocytic leukemia

Drug	Study No.	Dosage (mg/m², frequency)	Total No. of patients	No. of patients treated longer than 21 days	No. of patients achieving*—	
					CR	PR
6-MP	1	800, once weekly	21	16	—	—
		200–400, twice weekly	16	11	—	2
	2	800, twice weekly	25	15	2	1
		1600, twice weekly	5	4	1	1
		Total	67	46	7	
Methotrexate	1	40–60, once weekly	19	11	1	1
		20–30, twice weekly	18	12	—	1
	2	40, twice weekly	25	15	1†	3
		60, twice weekly	4	3	—	—
		Total	66	41	7	

*CR = complete remission; PR = partial remission.
†This patient also received prednisone during the drug trial with methotrexate.

TABLE 2.—Response in adults with acute lymphocytic or acute unclassified leukemia

Drug	Study No.	Dosage (mg/m², frequency)	Total No. of patients	No. of patients treated longer than 21 days	No. of patients achieving*—	
					CR	PR
6-MP	1	800, once weekly	4	3	—	—
		200–400, twice weekly	4	4	2	1
	2	800, twice weekly	5	4	1†	—
		1600, twice weekly	1	1	1†	—
		Total	14	12	5	
Methotrexate	1	40–80, once weekly	3	2	1	1
		20–30, twice weekly	3	2	—	2
	2	40, twice weekly	5	3	1	1
		60, twice weekly	1	—	—	—
		Total	12	7	6	

*CR = complete remission; PR = partial remission.
†This patient also received prednisone during the drug trial with 6-MP.

showed early evidence of improvement but required from 5 to 13 weeks to reach remission status. Remission lasted from 6 to 20 weeks (median, 15 weeks). In all patients who had marrow response there was also improvement to a partial or complete remission status of the peripheral blood, physical signs, and symptoms.

Methotrexate given once or twice weekly led to three responses in 37 patients with AML and four responses in six patients with ALL. Definite marrow improvement occurred 4–11 weeks after the start of treatment. Several marrow remissions were very brief, lasting only 2 weeks, although the peripheral blood response lasted longer. The longest methotrexate response lasted 13 weeks (median, 7 weeks).

The incidence of toxicity requiring dose modification was low. Only methotrexate given twice weekly produced toxicity regularly, suggesting that a higher drug dose could have been tolerated in all other groups. Thus, a quarter of those patients treated for more than 3 weeks with 6-MP twice weekly never had a wbc less than 2000 cells/mm³, marrow hypocellularity, or any evidence of gastrointestinal ulceration. Unexpected evidence of toxicity, consisting of chills and fever plus severe nausea and vomiting, was noted in five of the 45 patients who received 6-MP iv. These effects occurred repeatedly in some patients and were not related to the presence of pyrogens in any drug lot, but seemed to represent individual sensitivity to 6-MP as has been described previously (6–8).

The responses to the cross-over drug trial were even poorer. Eighteen patients received methotrexate and 17 received 6-MP as the second drug. Two patients responded with a partial remission after the alternate drug was given (one from each group), and two improved without reaching remission status. Both responders had also responded in the first drug trial.

Study No. 2

Seventy-one patients were treated in the second study. Fifty-nine had AML and 12 had either ALL or AUL. Five others were excluded from the study after randomization due to ineligibility or major deviations from the protocol.

Adrenal cortical steroids were given to 23 patients (15 with AML) at some time during the drug trial. Fifteen of them received the drug only at the end of the study. Three of the steroid-treated patients achieved remission, but the sequence of events in these three individuals suggested that the remissions resulted from the antimetabolite therapy. These cases, therefore, were included in the 71 analyzed.

The response rates in study No. 2 (tables 1 and 2) did not differ significantly from those noted in the first study. In patients with AML there was a 10% complete remission rate and a 7% partial remission rate with 6-MP treatment, while methotrexate treatment resulted in a 3% complete remission rate and a 10% partial remission rate. The 6-MP response rate was 26% for those patients with AML who were treated for more than 3 weeks.

Two of six patients with ALL or AUL treated with 6-MP had complete remissions and there were one complete and one partial remission among the six patients treated with methotrexate.

Marrow responses due to 6-MP could be demonstrated at 6–16 weeks, while remissions due to methotrexate were noted at 5–10 weeks. 6-MP responses lasted from 10 to 54 weeks (median, 25 weeks) and methotrexate responses lasted from 2 to 139+ weeks (median, 16 weeks).

Severe toxicity occurred in most patients receiving the higher doses (table 3). All 18 patients who received methotrexate at doses of 40 mg/m² twice weekly for at least 3 weeks and 84% of the 19 patients treated with 6-MP at doses of 800 mg/m² twice weekly for 3 weeks or more had significant side effects.

In study No. 2, 20 patients (10 who received 6-MP and 10 who received methotrexate in their original drug trials) received "cross-over" drug trials. Treatment with the alternate drug resulted in one complete remission, one partial remission, and one improvement for each cross-over treatment group. These responses occurred in patients who had had either remission or some hematologic improvement after the original drug was given. Thus, in the combined studies, responses to either agent given as the second drug occurred only in patients who had remitted or had had some hematologic improvement after receiving the first drug.

The effect of adrenal cortical steroids on the overall response rate was analyzed in both studies. With either drug and in both morphologic forms of the disease, the response rates in the patients who did *not* receive steroids were the same as or higher than those for the group as a whole. This undoubtedly reflects the fact that in half the instances where steroids were used they represented terminal therapy.

TABLE 3.—Toxic effects resulting from study No. 2 (ALGB 6411)

Toxic effects	No. of patients receiving—			
	6-MP (mg/m², twice weekly)		Methotrexate (mg/m², twice weekly)	
	800	1600	40	60
Total No. treated	30	6	30	5
Wbc <2000 cells/mm³	13	5	18	3
<1000 cells/mm³	11	2	10	2
Hypocellular marrow	12	3	4	1
Mouth ulcers	6	3*	22	5
Nausea, vomiting	4	—	10	2
Diarrhea	1	—	9	2
Chills, fever	1	—	—	—
Phlebitis	1	3	—	—
Arterial thrombosis	2	—	—	—

*Two patients had severe edema.

Analysis of the *duration* of remissions indicates no great difference between ALL and AML. Responses from methotrexate lasted a median of 9 weeks in ALL and 5 weeks in AML. The median duration of response with 6-MP was 18 weeks for ALL and 15 weeks for AML.

Survival from the time of diagnosis was calculated for all patients in whom there was either no treatment or less than 2 weeks of treatment before entry in these studies (figs 1 and 2). The survival of nonresponders is similar for AML and ALL plus AUL. The occurrence of a complete or partial remission after treatment with either drug, however, was associated with significant and equivalent prolongation of survival for both types of leukemia. Because nearly 50% of the patients with ALL and AUL responded, their longer survival times influence the survival of the total population of ALL and AUL patients (fig 2). This

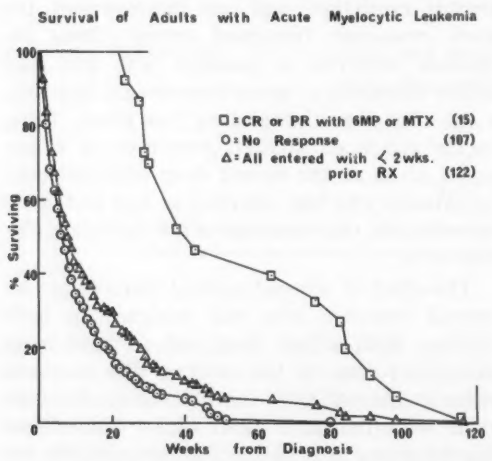
Survival of Adults with Acute Myelocytic Leukemia
□ = CR or PR with 6MP or MTX (15)
○ = No Response (107)
△ = All entered with < 2wks. prior RX (122)
% Surviving
Weeks from Diagnosis

FIGURE 1.—Survival time of adults with acute myelocytic leukemia.

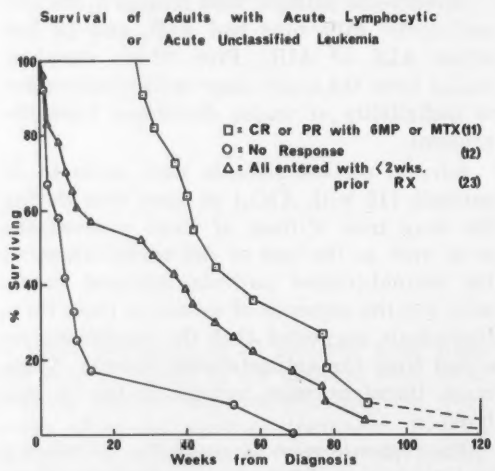
Survival of Adults with Acute Lymphocytic or Acute Unclassified Leukemia
□ = CR or PR with 6MP or MTX (11)
○ = No Response (12)
△ = All entered with < 2wks. prior RX (23)
% Surviving
Weeks from Diagnosis

FIGURE 2.—Survival time of adults with acute lymphocytic or acute unclassified leukemia.

was not the case for the total population of AML patients because the overall remission rate was only 12%.

Among those patients not previously treated, there was no survival-time advantage related to the first drug used for either the responders or the nonresponders.

DISCUSSION

No important prognostic signs other than the morphologic classification of the leukemia were identified in the group of patients treated. The development of remission in acute leukemia is an important factor in the survival time and the quality of life of the individual affected. The frequency of response in this study must therefore be compared with results previously obtained with the same drugs given orally to adults with acute leukemia (table 4). In AML no difference was noted between results of the current study in which the drugs were given iv and older studies in which either 6-MP or methotrexate was given in daily oral doses.

Huguley et al (9) reported the increased ef fectiveness of methotrexate when the drug was given in multiple small oral doses. Four doses per day in repeated 5-day courses produced a 32% complete remission rate plus an 11% partial remission rate in 19 patients with AML. However, in a randomized study reported by Vogler et al (10), the same observers found that the dose regimen produced only one partial remission in 14 patients with AML who received methotrexate as the first agent in the study, and one complete and one partial remission in the eight patients who received methotrexate as a "switch-over" drug. There was also one partial remission among eight patients who received methotrexate on a nonrandom basis after previously having received 6-MP. This gives a total complete plus partial remission rate of 13% in the 30 patients with AML in Vogler's study, and this result is not strikingly different from the figures cited in table 4.

In patients with AUL and ALL, the results with 6-MP were unchanged by the intermittent use of the drug given iv. The importance of the increase in response rate (complete plus partial remission) from 5%–14% in previous studies to 50% in this study in adults with ALL or AUL who received intermittent therapy with methotrexate given iv, cannot be interpreted in view of the small sample involved.

The effectiveness of 6-MP in adults with AML or ALL and AUL has apparently not been altered by the manipulation of the dose and schedule of 6-MP reported herein. This is consistent with results from remission maintenance in children, where 6-MP given twice weekly iv, in doses comparable to those used in these studies, was not superior to daily oral treatment (11). The rate of response to 6-MP in adults with AML remains lower than in

TABLE 4.—Results of studies Nos. 1 and 2 compared with previously obtained results

Study	Frequency of treatment	Treatment route	No. treated				Complete + partial remissions (%)			
			6-MP		Methotrexate		6-MP		Methotrexate	
			ALL + AUL	AML	ALL + AUL	AML	ALL + AUL	AML	ALL + AUL	AML
Studies Nos. 1 and 2	Once or twice weekly	iv	14	67	12	66	36	10	50	11
	Twice weekly only	iv	10	46	9	47	50	15	44	11
Unpublished data*	Daily	oral	41	167	20	70	34	22	5	11
ALGB Study II†	Daily	oral	11	31	7	34	36	16	14	6

*See footnote 5.
†See reference 1.

adults with ALL. The response of adults with ALL to 6-MP in this study (29% complete remission 7% partial remission) and in older studies where 6-MP was given orally daily (1),[†] is similar to that seen in children (1) with ALL treated with 6-MP given orally daily.

When these studies were begun, several concepts that have become important in the effective management of ALL in children were undergoing early exploration. It was recognized that some drugs are more useful in remission induction while others are best for remission maintenance. The studies reported here, however, involved induction of remission in adults, most of whom had AML, because no technique of inducing remission was sufficiently effective to allow studies during maintenance therapy. The more recent ability to produce remissions in approximately 50% of adults with AML (12,13) now provides an opportunity for the study of various maintenance regimens. Therefore, the present data are reported to provide additional clinical pharmacologic information on the use of intermittent 6-MP and methotrexate given alone. Intermittent administration of these drugs alone or in combination as part of a maintenance regimen in adults with AML is worth exploration.

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[†] *See* footnote 5.

Cyclophosphamide (NSC-26271), Vincristine (NSC-67574), Cytosine Arabinoside (NSC-63878), and Prednisone (NSC-10023) (COAP) Combination Chemotherapy for Acute Leukemia in Adults [1,2,3]

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SUMMARY

Sixty adults with acute leukemia were treated with a combination of vincristine, prednisone, cytosine arabinoside, and cyclophosphamide (COAP). Thirty-three of the 60 patients (55%) responded to treatment; 26 of the 46 patients (57%) who received an adequate trial of chemotherapy achieved a complete remission. Patients with acute myelocytic leukemia responded as well as patients with acute lymphocytic leukemia. Complete remission occurred in 23 of the 32 patients (72%) less than 50 years old but in only three of the 28 patients (11%) more than 50 years old. The median duration of the complete remissions was 43 weeks and the median survival time for patients who had complete remissions was 82 weeks. The most common toxic effects were myelosuppression and abnormalities of liver function.
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[2] Supported in part by grants CA-10376, CA-05831, CA-03754, and CA-10042 from the National Cancer Institute (NCI), National Institutes of Health, Public Health Service.

[3] Cyclophosphamide: 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate; Cytoxan.

Vincristine: leurocristine, sulfate (1:1).

Cytosine arabinoside: cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride; ara-C.

Prednisone: pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-.

Drugs used in this study were supplied by the Cancer Therapy Evaluation Branch, Division of Cancer Treatment, NCI.

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The introduction of cytosine arabinoside into clinical use against acute leukemia in adults marked a major advance in the treatment of that disease. Cytosine arabinoside, a pyrimidine nucleoside analog, is active in several animal tumor systems (1,2). It is effective in the treatment of both acute myelocytic leukemia and acute lymphocytic leukemia in man (3–5). When cytosine arabinoside was given by continuous infusion in 5-day courses, responses occurred in 39% of adults with acute myelocytic leukemia (6).

The therapeutic efficacy and the toxicity of cytosine arabinoside in mice with L1210 leukemia were greatly influenced by the schedule of administration (7). Although the drug prolonged the life of mice with far-advanced disease, it was curative only during the early stages of disease; however, combinations of cytosine arabinoside and another agent, such as BCNU, CCNU, cyclophosphamide, or 6-thio-

guanine,[7] were synergistic and curative even against far-advanced L1210 leukemia (8).

The encouraging results in studies with L1210 leukemia prompted a study of treatment with the combination of cytosine arabinoside and cyclophosphamide in adults with acute leukemia (9). This regimen produced a response rate of 53% in the 40 patients studied. In an effort to improve these results, vincristine and prednisone were added to the regimen (COAP). Since these two agents cause only minimal myelosuppression, it was possible to include them without altering the dosage of either cytosine arabinoside or cyclophosphamide. Furthermore, these drugs were found to be synergistic when they were administered in combination with 6-mercaptopurine and methotrexate (10),[8] and it was anticipated that a similar effect would be obtained if they were given with cytosine arabinoside and cyclophosphamide. The results obtained in our study of COAP therapy in 60 adults with acute leukemia indicate that this regimen produces responses in over 50% of the patients treated.

MATERIALS AND METHODS

Patients with acute leukemia whose bone marrow contained at least 30% blast cells and promyelocytes were eligible for COAP therapy, provided they had received no prior therapy with cytosine arabinoside or cyclophosphamide. Sixteen patients had received prior therapy; for most patients, various combinations of vincristine, prednisone, 6-mercaptopurine, and methotrexate had been used. Eight patients received COAP treatment while they were in a protected-environment unit (11).

The first 37 patients received COAP treatment in 4-day courses, but, as experience was gained with this regimen, therapy was prolonged to 5 days; thus, the remaining 23 patients received 5-day courses of treatment. When the drugs were administered in a 4-day course, the usual initial dose of each drug was as follows: vincristine, 2 mg given intravenously (iv) on Day 1 only; prednisone, 200 mg/day given orally in four divided doses; cytosine arabinoside, 120 mg/m²/day; and cyclophosphamide, 120 mg/m²/day (*see* table 1). The latter two drugs were given by rapid iv injection in divided doses every 8 hours. When the drugs were administered in a 5-day course, the usual initial dose of each drug was as follows: vincristine, 2 mg given iv on Day 1 only; prednisone, 200 mg/day given orally in four divided doses; cytosine arabinoside, 100 mg/m²/day; and cyclophosphamide, 100 mg/m²/day. Again, cytosine arabinoside and cyclophosphamide were given by rapid iv injection in divided doses every 8 hours. Subsequent courses were started 14 days after the beginning of the preceding course (9–10 days after the end of the preceding course) if there were no signs of progressive toxicity. Therapy was resumed earlier if there was rapid recovery of blast cells in the peripheral blood. The second course usually was begun at this time even if myelosuppression from the first course was still present. The dosage for subsequent courses of therapy was based upon the patients' responses to the first course. The dosage of cytosine arabinoside and cyclophosphamide was reduced by 25%–35% for patients whose marrow became hypocellular with a major reduction in abnormal cells. The dosage remained the same for patients who had substantial bone marrow improvement. The dosage was increased by 20% or more for patients whose marrow showed no improvement. The 1:1 ratio of cytosine arabinoside and cyclophosphamide was always maintained. The dosage of vincristine and prednisone always remained constant. Courses of therapy were continued at 2-week intervals until remission occurred or until there was evidence of progressive disease.

Bone marrow specimens were obtained before the administration of each course of therapy. Peripheral blood cell counts were determined at least three times weekly during the remission-induction period. Studies of hepatic and renal function were usually performed at weekly intervals.

Patients were considered to have had complete remissions if their bone marrow contained less than 5% blast cells and if their peripheral blood absolute granulocyte and platelet counts

[7] BCNU: NSC-409962; urea, 1,3-bis(2-chloroethyl)-1-nitroso-.

CCNU: NSC-79037; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-.

6-Thioguanine: NSC-752; purine-6-thiol, 2-amino-.

[8] 6-Mercaptopurine: NSC-755; purine-6-thiol, monohydrate.

Methotrexate: NSC-740; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-.

TABLE 1.—Cytosine arabinoside and cyclophosphamide treatment schedule

Total dose* (mg/m²)	No. of patients receiving course No.—					
	1	2	3	4	5	6
240–275	3	0	1	0	1	2
300–350	1	1	0	4	5	5
360–380	6	5	6	4	1	1
400–460	0	5	6	6	8	8
480–500	43	22	16	11	9	7
520–575	1	1	0	1	1	1
600–650	6	13	8	6	2	1
700–800	0	5	3	5	0	0
900–1250	0	2	4	2	2	0
1500–1750	0	0	1	2	1	0
Total	60	54	45	41	30	25

*Total dose of each drug per course.

were at least 1000 cells/mm³ and 100,000 cells/mm³, respectively, after recovery from the hematologic toxicity of the COAP regimen. Patients were considered to have had partial remissions if their bone marrow contained 6%–25% blast cells and the granulocyte and platelet counts in the peripheral blood were at the levels stated previously. A normal hemoglobin value was not a criterion for remission because cytosine arabinoside causes megaloblastic anemia in some patients (12).

Patients who had had remissions received maintenance treatment with COAP. Courses usually were administered at 3-week intervals. Either vincristine, prednisone, or cyclophosphamide was deleted from maintenance therapy when major toxicity occurred. The dose of cytosine arabinoside and cyclophosphamide for the first remission-maintenance course was 75% of the dose used for the last remission-induction course. During subsequent courses, the drugs were given at a dose sufficient to reduce the absolute granulocyte count to approximately 1000 cells/mm³ or the platelet count to less than 100,000 cells/mm³ or both. More intensive chemotherapy was given if examination of the bone marrow revealed evidence of progressive disease.

RESULTS

Response

Sixty adults (32 men and 28 women) with acute leukemia received COAP therapy between September 23, 1968, and January 21, 1971. The median age was 48 years, with a range of 15–82 years. Thirty-nine patients had acute myelocytic or acute monocytic leukemia (AML) and 21 patients had acute lymphocytic or acute undifferentiated leukemia (ALL).

Forty-six of the 60 patients (77%) survived long enough to receive an adequate trial of chemotherapy, which was considered to be more than two courses of therapy (table 2). Thirty-three of the 60 patients (55%) responded favorably to COAP treatment and 26 (43%) had complete remissions. If only the 46 patients who received an adequate trial are considered, the

TABLE 2.—Response to COAP therapy

	No. of patients
Entered study	60
Adequate trial	46
Complete remission	26
Partial remission	5
Hematologic improvement	2
Treatment failure	13

response rate was 72% and the complete remission rate was 57%.

The response to COAP therapy was related to the age of the patients (table 3). The proportion of the patients treated initially who survived long enough to receive an adequate trial of chemotherapy was larger in the group of patients less than 50 years old than in the group of patients more than 50 years old (29 of 32 patients or 91% vs 17 of 28 patients or 61%). The complete remission rate was 72% for the younger group (23 of 32 patients treated) compared with only 11% for the older group (three of 28 patients treated).

Morphologic diagnosis was not an important prognostic factor. The proportion of patients who received an adequate trial of chemotherapy was slightly higher in the group with ALL (17 of 21 patients or 81%) than in the group with AML (29 of 39 patients or 74%). Nine of the 21 patients (43%) with ALL had complete remissions compared with 17 of the 39 patients (44%) with AML. Only eight of the patients with ALL were more than 50 years old and none of the eight had a complete remission, whereas 20 of the 39 patients with AML were in this age group. Considering the two age groups separately, the complete remission rate for patients with ALL is similar to that for patients with AML. Hence, age is a more important prognostic factor than morphologic diagnosis.

Fifteen of the 16 patients (94%) who had received prior therapy survived long enough to receive an adequate trial of COAP therapy compared with 31 of the 44 patients (70%) who had received no prior therapy. Ten patients (63%) who had received prior therapy responded favorably to COAP therapy compared with 23 patients (52%) who had not received prior therapy; however, considering only those patients who received an adequate trial of COAP, the complete remission rates were 47% and 61%, respectively.

All of the eight patients treated in a protected-environment unit received an adequate trial of COAP therapy. Five patients had complete remissions, two had partial remissions, and one failed to respond. All of these patients were less than 60 years old. The complete remission rate for these patients was 63% compared with 55% for the 21 patients in the same age group who were treated in regular hospital rooms.

The median duration of complete remission was 43 weeks with a range of 2–142+weeks (fig 1). Seven patients are still in remission 22, 80, 83, 112, 121, 124, and 142 weeks from the start of response. The median duration of improvement short of complete remission was only 6 weeks with a range of 3–28 weeks.

The median number of courses of therapy given before complete remission occurred was two. Three of the 26 patients had complete remissions after one course of treatment, three patients required four courses, and one patient required five courses. The median total dose of cytosine arabinoside and cyclophosphamide given before remission occurred was 1280 mg/m^2 of body surface area (range, 480–5120 mg/m^2). During complete remission, the patients received a median of 13 courses of chemotherapy (range, one to 39 courses). The median total dose of cytosine arabinoside and cyclophosphamide given during remission maintenance was 4.6 g/m^2 (range, 320 mg/m^2–22.6 g/m^2).

Patients who responded to COAP therapy survived substantially longer than patients who failed to respond (fig 2). The median duration

TABLE 3.—Age, morphologic diagnosis, and response of patients treated with COAP

	No. of patients < 50 years old			No. of patients > 50 years old		
	AML	ALL	Total	AML	ALL	Total
Entered study	19	13	32	20	8	28
Adequate trial	16	13	29	13	4	17
Complete remission	14	9	23	3	0	3
Adequate trial/entered	84%	100%	91%	65%	50%	61%
Complete remission/entered	74%	69%	72%	15%	0	11%
Complete remission/adequate trial	88%	69%	79%	23%	0	18%

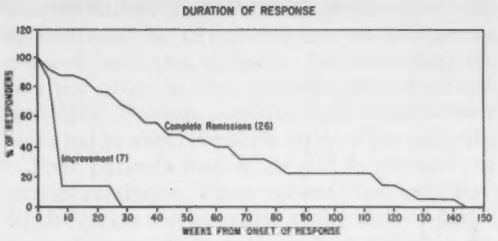

FIGURE 1.—Duration of response to COAP therapy. Numbers in parentheses indicate numbers of patients. Duration of response is dated from onset of response not from start of therapy.

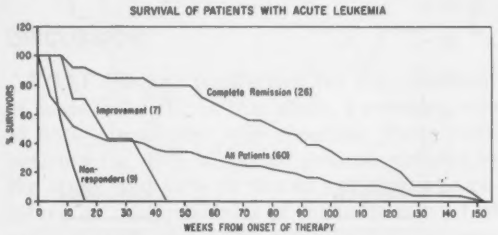

FIGURE 2.—Duration of survival of patients with acute leukemia after COAP therapy. The 14 patients who did not receive an adequate trial are not included in the group of nonresponders.

of survival for all patients treated with COAP was 14 weeks. The median survival time of patients achieving complete remission was 82 weeks. Nine of these patients are still alive 36, 86, 89, 100, 102, 122, 126, 126, and 148 weeks from the start of therapy. The seven patients who responded favorably but did not have complete remissions had a median survival time of 22 weeks. The nine patients who received an adequate trial but failed to respond had a median survival time of only 8 weeks.

Patients with ALL had longer complete remissions and survival times (fig 3). The median duration of survival from the start of therapy was 22 weeks for patients with ALL, compared with 11 weeks for patients with AML. The median duration of complete remission was 58 weeks for patients with ALL compared with 42 weeks for patients with AML. Age was more important in predicting the duration of survival than morphologic diagnosis since fewer elderly patients had remissions. The median duration of survival for patients less than 50 years old was 60 weeks (range, 1–148+ weeks) compared with only 6 weeks (range, 1–72 weeks) for patients more than 50 years old.

Toxic Effects

Moderate to severe myelosuppression occurred regularly after COAP therapy. Figure 4 illustrates the effect of initial and subsequent courses of COAP (total doses of cytosine arabinoside and cyclophosphamide, 480–500 mg/m²) on the white blood cell count (wbc). The median wbc nadir was 1100 cells/mm³ (range, 200–6500 cells/mm³) during the first course and 900 cells/mm³ (range, 0–4300 cells/mm³) during subsequent courses. Figure 5 illustrates the effect of initial and subsequent courses of COAP (total doses of cytosine arabinoside and cyclophosphamide, 480–500 mg/m²) on the platelet count. The median platelet count nadir was

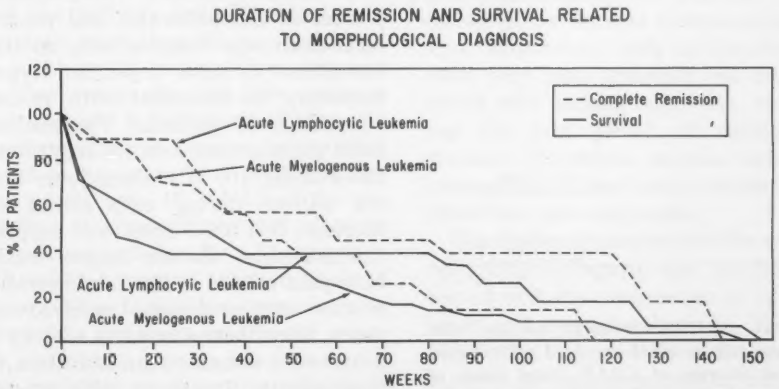

FIGURE 3.—Duration of complete remission and duration of survival of patients with ALL compared with patients with AML.

31,000 cells/mm³ (range, 1000–89,000 cells/mm³) during the first course and 18,000 cells/mm³ (range, 4000–139,000 cells/mm³) during subsequent courses. Often, subsequent courses were started before the peripheral blood cell counts had recovered from the previous course. When patients had remissions, the peripheral blood cell counts often continued to increase after the next course was begun, as is demonstrated by the platelet counts in figure 5. There was no evidence of increasing myelosuppression with increasing doses of the drugs, although the recovery period was more prolonged. Some patients were more sensitive to the chemotherapy than others and the dosage had to be adjusted for each patient. Adjustments were based on a patient's response to prior courses of therapy.

Hepatitis developed in ten patients during COAP therapy. Septicemia was the cause of the hepatitis in five patients. In the remaining five patients, it was not possible to determine whether the hepatitis was due to treatment or to viral infection transmitted by the transfusion of blood products. However, 26 patients had elevated serum glutamic oxaloacetic transaminase (SGOT) values, 23 patients had elevated serum glutamic pyruvic transaminase (SGPT) values, and 17 patients had elevated serum bilirubin values. The median highest value for SGOT was 129 units (range, 64–3200 units),

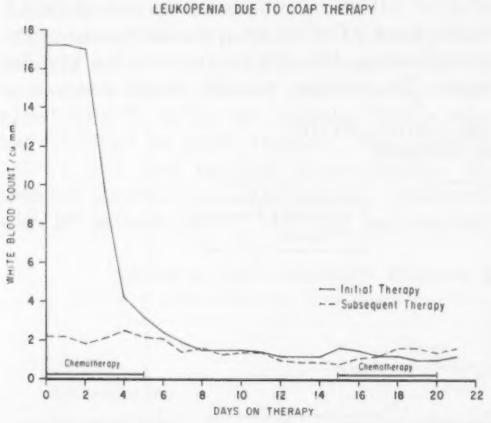

FIGURE 4.—Effect of COAP therapy on the wbc. Changes in wbc counts which occurred after initial and subsequent courses of COAP (total doses of cytosine arabinoside and cyclophosphamide, 480–500 mg/m²) are shown. There were 40 evaluable initial courses and 48 evaluable subsequent courses.

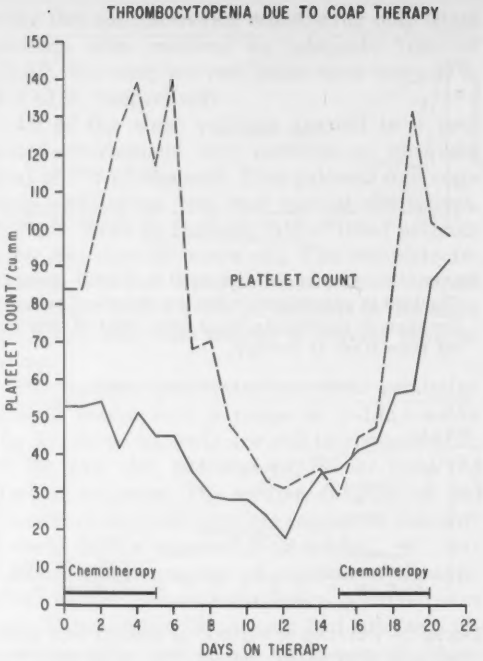

FIGURE 5.—Effect of COAP therapy on the platelet count. Changes in platelet counts which occurred after initial and subsequent courses of COAP (total doses of cytosine arabinoside and cyclophosphamide, 480–500 mg/m²) are shown.

the median highest value for SGPT was 162 units (range, 54–1400 units), and the median highest value for serum bilirubin was 4.2 mg/100 ml (range, 1.2–19.6 mg/100 ml). These abnormalities occurred intermittently after courses of chemotherapy, and progressive deterioration was observed only in the patients considered to have hepatitis. Hyperglycemia secondary to treatment with prednisone developed in 11 patients. The median highest blood glucose value was 196 mg/100 ml (range, 150–513 mg/100 ml). Drug fever developed in one patient during each course of chemotherapy; this toxic effect was suppressed with antihistamines. Severe muscle weakness and bone pain related to the administration of vincristine and prednisone occurred in two patients. Paresthesias and loss of deep tendon reflexes were common manifestations of vincristine toxicity. Treatment with vincristine was discontinued for 16 patients during remission maintenance because progressive neurologic

toxicity was evident. Prednisone treatment was discontinued for 12 patients and the dosage was reduced for three patients. Cyclophosphamide was not given to four patients after hepatitis developed. Nausea, vomiting, and alopecia were observed in approximately 20% of the patients.

Four patients died while still in complete or partial remission. Three patients had both serious infection and hepatitis at the time of death. Two of these three patients had adequate peripheral blood cell counts. Severe pancytopenia secondary to COAP therapy developed in the fourth patient; this patient later died after infection occurred.

DISCUSSION

COAP therapy is effective for the treatment of acute leukemia; in this study, a response rate of 55% (in adults) was observed. Twenty-six patients (ie, 43% of the 60 patients entered in the study and 57% of the 46 patients who received an adequate trial of chemotherapy) had complete remissions. The response rate achieved with COAP is similar to that achieved with cytosine arabinoside used in combination with cyclophosphamide or 6-thioguanine (9,13); however, the median duration of remission was 10¾ months for patients receiving COAP compared with 6 months for patients receiving cytosine arabinoside and cyclophosphamide or 6-thioguanine. Four patients treated with COAP are still in remission at the end of 2 years.

The effect of age on response to therapy has been observed in other studies (9,14). Nearly one half of the patients initially included in this study were more than 50 years old and only three of these 28 patients had complete remissions. One reason for the poor response among these patients is that many of them die of complications of their diseases before they have received an adequate trial of chemotherapy. In this study, only 17 (61%) of the patients more than 50 years old received adequate trials; of the 32 patients less than 50 years old, 29 (more than 90%) received adequate trials of chemotherapy. Other factors are operative also since the complete remission rate in the group of patients receiving adequate trials was only 18% (three patients) in the group of elderly patients compared with 79% (23 patients) in the groups of patients less than 50 years old. Elderly patients have decreased bone marrow reserves and may be incapable of adequate recovery after myelosuppressive chemotherapy.

Morphologic diagnosis was a less important factor than age in patient response to COAP therapy; this was true also for chemotherapy with cytosine arabinoside and cyclophosphamide (9). Of the 39 patients with AML, 17 (44%) had complete remissions compared with nine (43%) of the 21 patients with ALL.

In our previous study, patients with ALL treated with cytosine arabinoside and cyclophosphamide had much shorter remissions than patients with AML (9). The median durations of the complete remissions were 2¼ and 8 months, respectively; however, the median duration of the complete remissions after COAP therapy was 14½ months for patients with ALL and 10½ months for patients with AML. The substantial increase in remission duration in patients with ALL was probably due to the addition of vincristine and prednisone in the COAP regimen.

Response to COAP therapy was also dependent upon whether patients had received prior antileukemic therapy; this has been observed previously (14). The response rate for the 16 previously treated patients was 63% compared with 52% for the group of previously untreated patients. More than 90% of the former patients survived long enough to receive an adequate trial of chemotherapy compared with 70% of the latter patients. Patients who had received prior chemotherapy were under constant observation and new therapy was begun promptly before the disease became far-advanced or serious complications developed. Poor responders, such as the elderly, patients with very high leukemic cell counts, and patients with serious infections, usually die during the first period of remission-induction therapy. Therefore, studies including a high proportion of previously treated patients include few poor responders.

The myelosuppressive toxicity which occurred with COAP therapy was similar to that observed with the combination of cytosine arabinoside and cyclophosphamide. The addition of vincristine and prednisone did not increase the severity of myelosuppression but it did introduce neurologic and metabolic abnormalities. These two agents often had to be deleted from

maintenance therapy because of their toxicity. Hepatitis occurred in ten patients and more than 35% of the patients had evidence of drug-induced hepatic toxicity. The hepatitis usually occurred either in association with serious bacterial infection or during the period appropriate for the development of viral hepatitis transmitted by blood transfusion. Consequently, it was difficult to determine how often the chemotherapy induced hepatitis. The abnormalities of liver function occurred regularly after each course of therapy in some patients and sporadically in others. The importance of the toxicity of the COAP therapy is evident since four patients died while in remission.

The four-drug combination of vincristine, prednisone, cytosine arabinoside, and cyclophosphamide is an effective therapeutic regimen for adults with acute leukemia. It is active against both AML and ALL and produces remissions lasting more than 10 months. The results of this study have led the Southwest Cancer Chemotherapy Study Group to embark on a study comparing COAP treatment with cytosine arabinoside treatment administered according to the same schedule.

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Effect of Resection of the Primary Neoplasm on Responsiveness to Chemotherapy of Patients With Large Bowel Cancer [1,2]

C. G. Moertel, R. J. Reitemeier, A. J. Schutt, and R. G. Hahn [3]

SUMMARY

In patients with large bowel cancer, the presence or absence of the primary tumor had no detectable influence on the objective response to chemotherapy with the fluorinated pyrimidines, 5-fluorouracil and 5-fluoro-2′-deoxyuridine, or the nitrosourea derivatives, BCNU and CCNU.

[Cancer Chemother Rep 56:551–552, 1972]

Animals with pulmonary metastasis of Lewis lung tumor have had enhanced responsiveness to chemotherapy after resection of the limb containing the primary transplant (1). In man, there have been occasional regressions of metastatic foci after resection of the primary neoplasm. Therefore, it seemed appropriate to compare the chemotherapeutic responsiveness of patients who had metastatic large bowel carcinoma with unaltered or locally recurrent primary tumors with that of patients whose primary tumors had been completely resected without apparent evidence of local recurrence. A perceptible treatment advantage for patients with resected neoplasms would be important in stratification of patients for chemotherapy trials. A strong argument could also be made for palliative resection of the primary lesion before chemotherapy was attempted.

MATERIALS AND METHODS

All patients selected for study had histologically confirmed adenocarcinomas originating in the colon or rectum, and all had measurable areas of malignant disease to serve as indicators of response to therapy.

Three hundred patients were treated with the fluorinated pyrimidines: 153 with 5-fluorouracil (5-FU; NSC-19893) and 147 with 5-fluoro-2′-deoxyuridine (5-FUDR; NSC-27640), each given by rapid intravenous (iv) injection in intensive courses according to previously described methods (2). Two courses were administered and the patients were evaluated for response at 10 weeks.

A total of 135 patients were treated with the nitrosourea derivatives: 69 with 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU; NSC-409-962) and 66 with 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU; NSC-79037). BCNU was administered in total doses ranging from 250 to 275 mg/m^2 given iv in divided-dose courses over 3-6 consecutive days. CCNU was administered in a single oral dose of 130 mg/m^2. Nitrosourea-treated patients were evaluated for response at 8 weeks.

Patients were considered to have an objective response if they demonstrated a greater than 50% reduction in the product of the longest perpendicular diameters of the most clearly measurable area of known malignant disease. There could be no increase in other areas of malignant disease, and no new areas of malignant disease could appear.

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[2] Supported in part by contract NIH-70-2066 from Chemotherapy, National Cancer Institute, National Institutes of Health.

[3] Division of Gastroenterology and Internal Medicine (Drs. Moertel, Reitemeier, and Schutt) and Division of Clinical Oncology and Internal Medicine (Dr. Hahn), Mayo Clinic and Mayo Foundation, Rochester, Minn.

TABLE 1.—Relationship of the presence of the primary tumor to the responsiveness to chemotherapy of patients with large bowel cancer

Drug	Status of primary tumor	Patients treated	Objective responses (%)
Fluorinated pyrimidines: 5-FU or 5-FUDR	Unaltered or locally recurrent	89	23.6
	Resected, no local recurrence	211	22.8
Nitrosoureas: BCNU or CCNU	Unaltered or locally recurrent	58	10.3
	Resected, no local recurrence	77	11.7

RESULTS

The objective response rates for treatment with fluorinated pyrimidines and nitrosoureas are related in table 1 to the presence or absence of the primary neoplasm. No detectable difference in responsiveness could be demonstrated. There was also no detectable difference when these figures were broken down according to the specific chemotherapeutic agents used within each major drug group or according to whether the primary lesion was unaltered or locally recurrent.

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ANNOUNCEMENTS

Tenth Symposium on Biomathematics and Computer Science in the Life Sciences

The University of Texas Graduate School of Biomedical Sciences at Houston, Division of Continuing Education, announces the Tenth Symposium on Biomathematics and Computer Science in the Life Sciences, which will be held March 29, 30, and 31, 1973, in Houston, Texas.

Topics for the session of the symposium will include mathematical, statistical, and computing applications in biology and medicine.

Papers of an interdisciplinary nature will be particularly welcomed by the Program Committee, as will appropriate papers on education. An Abstract of the paper must be submitted to the Chairman, Dr. Stuart O. Zimmerman, The University of Texas M. D. Anderson Hospital and Tumor Institute at Houston, 6723 Bertner Drive, Houston, Texas 77025, prior to December 15, 1972.

For further information write: Office of the Dean, The University of Texas Graduate School of Biomedical Sciences at Houston, Division of Continuing Education, P. O. Box 20367, Houston, Texas 77025.

Cancer Fellowships

Postgraduate Fellowships in cancer for practicing physicians are available at the University of Southern California School of Medicine and the Los Angeles County-University of Southern California Medical Center. These Fellowships are sponsored by the National Cancer Institute, are of 1 month's duration, and carry a stipend of $750. Separate programs are available in gynecologic oncology, medical oncology, radiation therapy, tumor pathology, and tumor surgery. Applicants should be Board eligible or Board certified and should submit a résumé of their professional background to Arthur J. Donovan, MD, Program Director, Cancer Training Program, School of Medicine, University of Southern California, 2025 Zonal Avenue, Los Angeles, California 90033.

Pediatric Oncology Fellowship

The Department of Pediatrics of the University of Texas at Houston, M. D. Anderson Hospital and Tumor Institute, has fellowships available in clinical pediatric oncology. For further information write to W. W. Sutow, MD, The University of Texas at Houston, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77025.

8th International Congress of Chemotherapy

On behalf of the International Society of Chemotherapy, the Hellenic Society of Chemotherapy invites all interested investigators to attend the 8th International Congress of Chemotherapy to be held in Athens, Greece in the Autumn of 1973.

The Scientific Programme of the Congress will be comprised of the presentation of papers covering 1) chemotherapy of bacterial infections, 2) chemotherapy of parasitic and mycotic infections, 3) chemotherapy of viral infections, 4) chemotherapy of cancer, and 5) new drugs, considered from the aspects of *a*) biochemistry, *b*) pharmacology, *c*) mode of action, *d*) resistance, *e*) clinical applications, *f*) laboratory control, *g*) experimental evaluation, and *h*) immunologic effects. A detailed Scientific Programme will be available later this year.

The official language of the Congress will be English.

An effort is being made to have the Congress in two phases. The first phase will be held in Athens (3 days), and the second on board, cruising the Aegean (4 days). While cruising, scientific sessions will take place in the afternoons and early evenings. In the mornings we will visit Crete, Rhodes, and the Asklepeion of Kos. The expenses for the cruise (full board) will not exceed the city expenses (counting hotels and meals) and an effort will be made to keep them at a minimum.

The Social Programme during the first phase of the Congress will give the participants the opportunity to attend an ancient tragedy performance at the Herodus Atticus Theatre at the foot of the Acropolis, to have guided tours of the Acropolis and other archaeological sites, and to visit various beaches. An opening cocktail party and a gala evening are also scheduled.

For accompanying members a special entertainment programme, including various excursions, is to be organized.

An early reply showing the interest of participants regarding this preliminary plan will enable us to proceed with its organization. For further information write to the 8th International Congress of Chemotherapy, PO Box 1554, Athens, Greece.

Medical Oncology Fellowships

A 1- or 2-year training program in Medical Oncology is offered at Tufts University School of Medicine and affiliated hospitals. Training is shared by the Medical Oncology units of the New England Medical Center, Lemuel Shattuck, and Pondville Hospitals. Elective rotations on Therapeutic Radiology, Surgical Oncology, Pathology, and Hematology are available. Fellowships require internship and 1 or 2 years of previous residency training. The program is sponsored by the National Cancer Institute and carries a stipend of approximately $14,000 per annum. Further information may be obtained from Larry Nathanson, MD, Chief, Medical Oncology Service, New England Medical Center Hospitals, 171 Harrison Avenue, Boston, Massachusetts 02111.

CORRECTION

Due to a printing error the following structure was omitted from page 335 of the June issue of *Cancer Chemotherapy Reports* (Vol. 56, No. 3, 1972).

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